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DEPARTMENT OF BIOLOGY

Contractor

Document Title

A STUDY OF THE DIFFUSSION OF METHYLENEDIANILINE IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM WITH ATTACHMENTS

Chemical Category

METHYLENEDIANILINE (101-77-9)

APPENDIX II

Document préparé par le
LABORATOIRE D'ETUDES DU METABOLISME DES MEDICAMENTS
Département de Biologie
Commissariat à l'Energie Atomique
France

Table I

time in hours / organ	24 ♀
kidneys: medullar zone	++++
kidneys: cortical zone	++
liver	++
large intestine	++
spleen	++
heart	++
salivary glands	++
skin	++
lungs	+
bone marrow	+
bladder	

Quantity of radioactivity localized by autoradiography in the rat.

++++ very strong

+++ strong

++ medium

+ weak

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Département de Biologie
Commissariat à l'Energie Atomique
France

SUMMARY

produits chimiques
UGINE KUHLMANN

CENTRE D'APPLICATION DE LEVALLOIS
Boîte postale 131
92303 LEVALLOIS-PERRET CEDEX

N/Réf.: 27 809 - GL. KUPKA-BODIN/DS

V/Réf.:

LEVALLOIS, LE 29th October 1976

TOXICOLOGY SUB-COMMITEE

APPENDIX II

ASSISTANCE TO THE CEA FOR EA4 PROGRAM :

TOLUENE DIISOCYANATE METABOLISM

DETERMINATION OF TDI CONCENTRATION IN THE AIR

WITH THE PCUK FLOWING ELECTROLYTE CELL

LABORATOIRES : 95, rue Danton
Téléphone : 758.12.55
Télex : 620 750

3, rue Collange
Téléphone : 270.03.10
Télex : 630 012

Société Anonyme au Capital de 375.000.000 F - Siège Social : 25, Bd de l'Amiral Bruix, 75116 PARIS - R.C. Paris B 542040258

2.2. Atmosphere control

Sampling was carried out by sucking-up from the rat box disposed in the enclosure of the TDI vapor generator.

The compressed air inlet was 10 mm above the cup containing 5 ml TDI. The temperature was 25°C.

The TDI contents of the rat box were measured versus compressed air pressure and versus time. Their recordings are given at the page A II 8.

The results are as follow :

Compressed air pressure	TDI concentration ppm vol	Remarks
0,2	0,25 to 0,30	
0,6	0,80 to 0,85	
0,8	idem	Important projection on the walls in 1/2 U
1,2	idem	
1,5	1	

It can be noted that, whatever the air pressure may be from 0,6 to 1,2 kg/cm2, the TDI concentration is constant, about 0,85 ppm vol.

On the other hand, 1,5 kg/cm2 pressure creates projections of TDI aerosols on the air inlet tube to the rat cage, which increase indeed the TDI vapor concentration in the air to about 10 ppm.

If the TDI amount in the cup is about 1 ml and not 5 ml, the obtained TDI concentrations are lower.

The time required for setting up a constant TDI concentration in the rat box is about 20 to 25 minutes, whatever the used air pressure.

3 - CONCLUSION

With the generator that the CEA have, it is possible to obtain atmospheres containing a constant concentration of about 0,8 to 0,9 ppm vol of TDI at 25°C.

The inlet of air compressed to 0,7 kg/cm2 pressure has to be fixed 10 mm above the cup containing the TDI.

A special care must be taken to blow off the device, as the TDI is absorbed by any plastic material : polypropylène, polyvinyl chloride ...
It is strongly advised to use isoversinic tubes.

The aim of these trials was to control the TDI content of the artificial atmosphere within the inhalation room used for rat contamination.
(The TDI vapor generator is given in the diagram 1)

1 - DETERMINATION PRINCIPLE

The flowing electrolyte cell is formed by a glass cell, two platine electrodes, one of which, the reference one is permanently immersed in an electrolyte that flows on the second sieve-shaped platine electrode.

The gas to be studied comes into the cell at the level of this sieve, called indicator electrode.

The electrochemical reaction between the gas and the oxidising solution at the indicator electrode level originates a micro-current between the two electrodes, which current is proportional to the TDI content of the gas.

The glass cell diagram is given on page A II 5.

2 - WORKING CONDITIONS

The gas is sucked in the cell by means of a pump. The 9 l/mn flow is measured and regulated with a flow-meter and a pressure regulator.

The measurement is sequential i.e. the 20 s admission period of the gas to be studied is followed by a 6 mn admission period of pure air, called reference air, that blows off the apparatus.

The electrolyte (concentrated H_2SO_4 + 100 mg/l I_2O_5) flows from a tank to the cell at about 40-60 ml/h and is collected at the cell outlet.

The two electrodes are connected to a recordind micro-amperemeter.

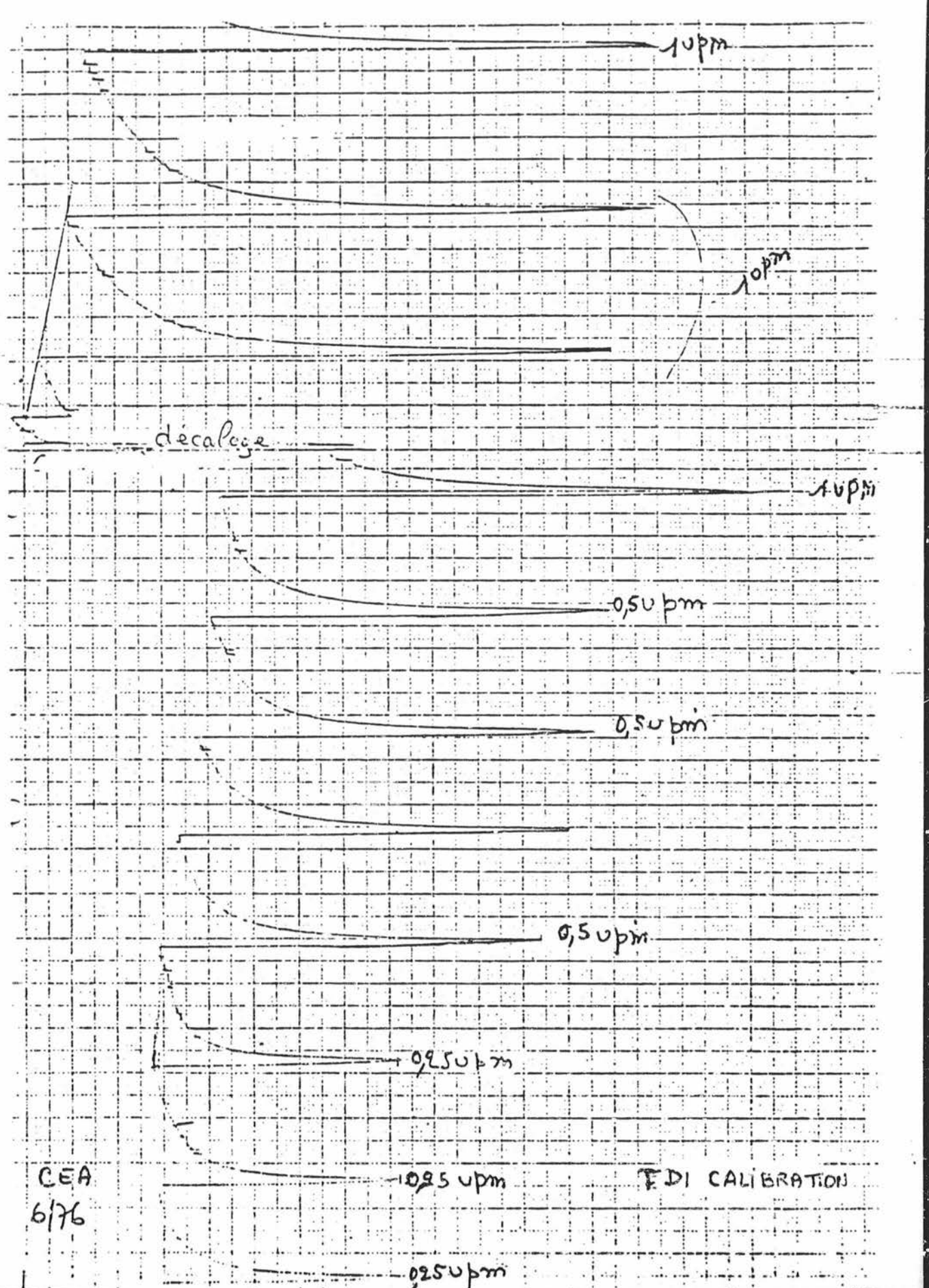
2.1. Calibrating

The device is calibrated with an air having known TDI contents from 0,25 to 1 ppm vol. These concentrations are obtained by a precise dilution of an air containing about 2 ppm vol. TDI which is obtained by sweeping the TDI surface in a tank (4,2 ppm vol. at 25

Recordings of TDI determination and TDI calibration curve are shown on pages A II 6 and A II 7.

.../...

RECORDINGS OF TDI DETERMINATION



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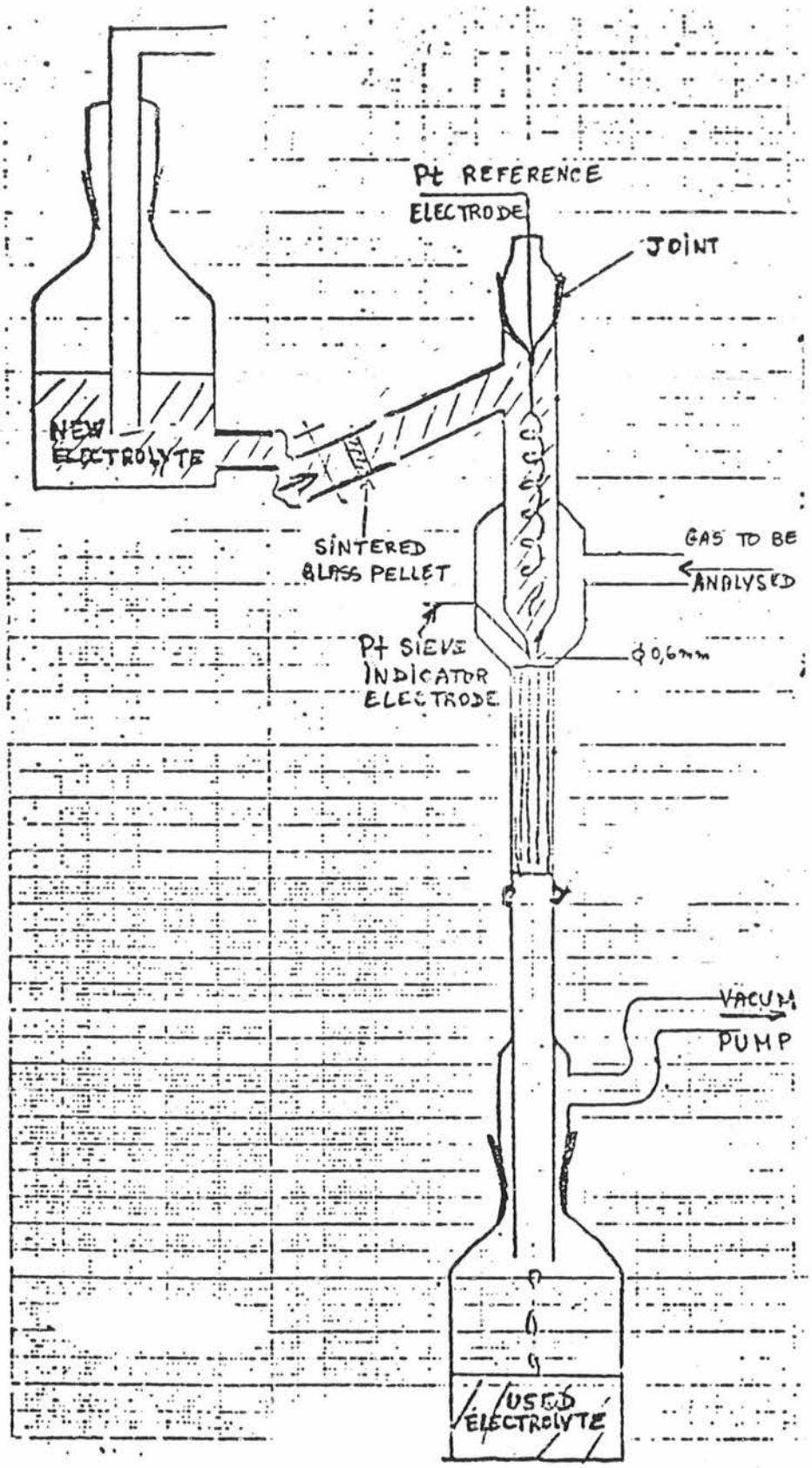
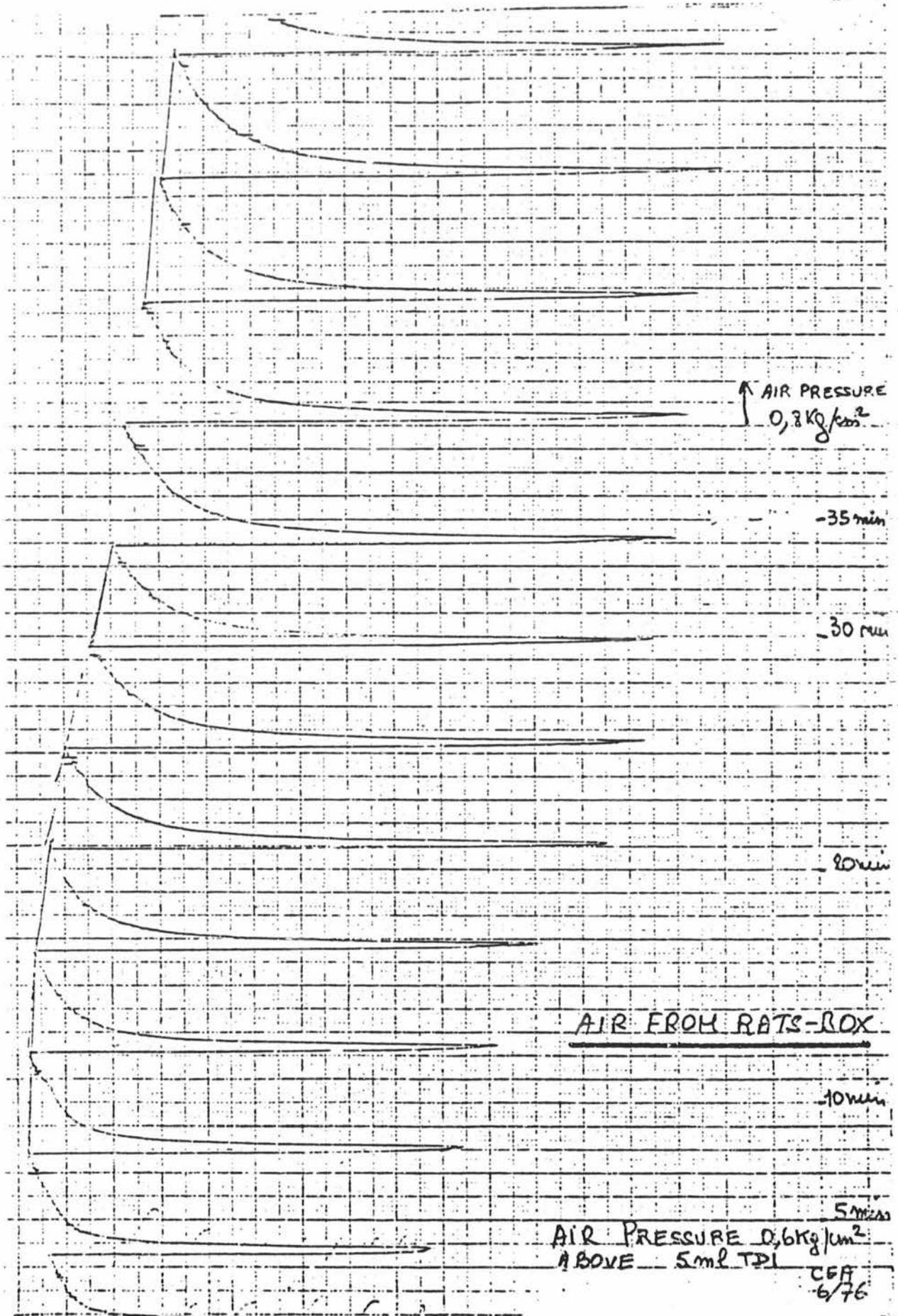
TDI CALIBRATION

DIAGRAM OF THE FLOWING ELECTROLYTE CELL



RECORDINGS OF AIR FROM RATS-BOX



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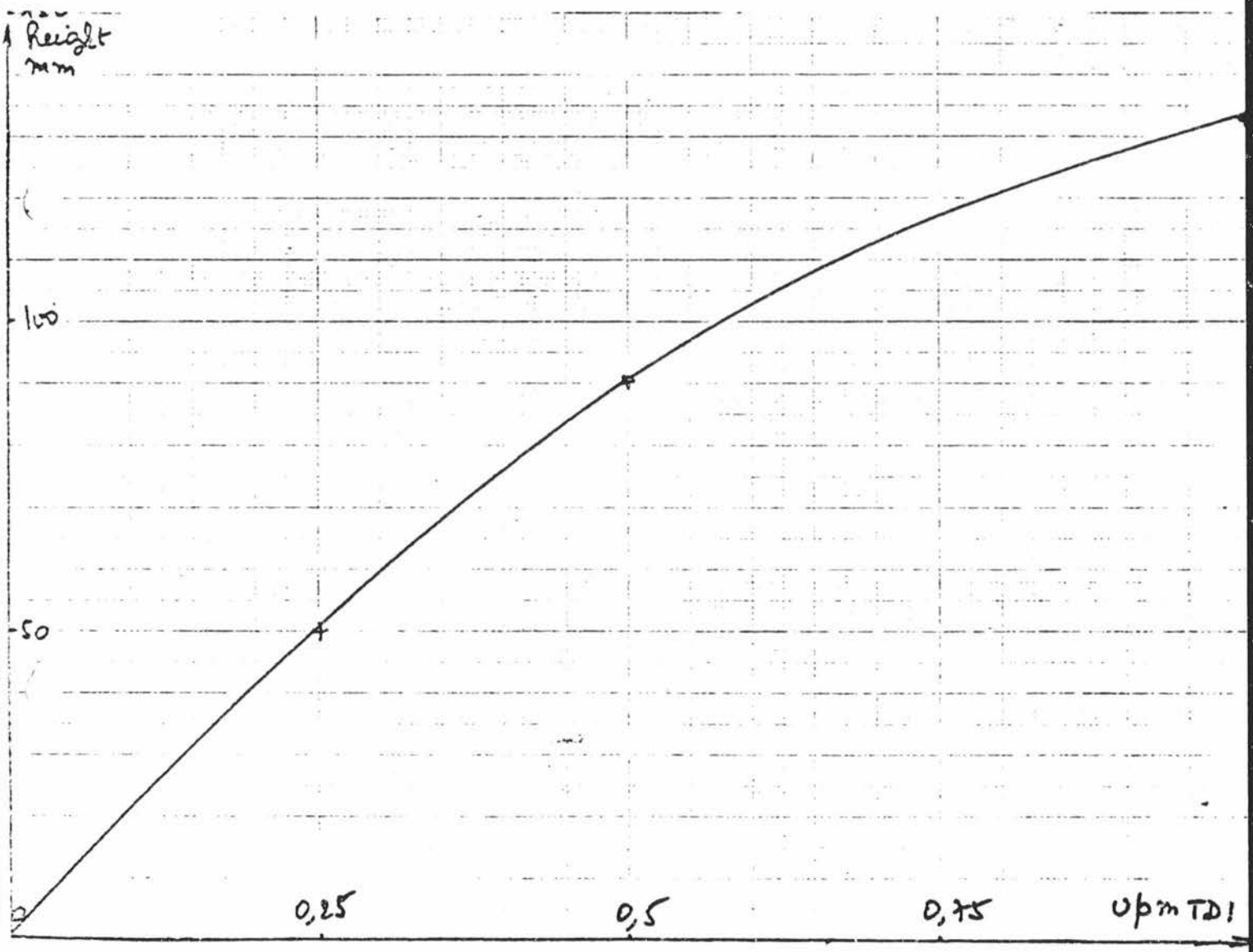
TDI CALIBRATION CURVE



N°	4	8	9	mean ± es
SEX	♂	♀	♂	
Dose (µc/Kg)	128.59	175.03	161.36	155.16
TIME (h)				
1	0.73	1.12	3.63	2.49 ± 0.91
2	1.74	1.12	8.93	3.93 ± 1.45
3	2.88	1.16	10.28	4.77 ± 1.62
4	4.66	1.15	11.15	5.65 ± 1.69
5	4.85	-	-	-
6	4.95	1.21	11.82	5.99 ± 1.79
8	5.70	1.45	11.98	6.38 ± 1.77
12	7.04	2.69	11.20	6.98 ± 1.42
24	8.40	4.60	9.62	7.54 ± 0.87
48	6.11	4.20	6.15	5.49 ± 0.37
72	3.86	2.84	4.26	3.65 ± 0.24
96	2.73	2.23	2.91	2.62 ± 0.12
120	-	1.48	1.89	1.68 ± 0.14
144	-	1.16	1.51	1.33 ± 0.12
168	-	0.89	1.05	0.97 ± 0.06
192	-	0.67	0.76	0.71 ± 0.03
216	-	0.51	0.59	0.55 ± 0.03
240	-	0.42	0.50	0.46 ± 0.03
264	-	0.32	0.35	0.33 ± 0.01
288	-	0.26	0.28	0.27 ± 0.01
312	-	0.21	0.25	0.23 ± 0.01
336	-	0.16	0.21	0.18 ± 0.02
360	-	0.13	-	-

TABLE I : Individual results showing the evolution of ^{14}C radioactivity in the blood of rats as a function of time (see table II) after intramuscular injection of TDI ^{14}C. The results are expressed as a function of the radioactivity injected into the rats, measured per gram of blood (‰/g). The mean for each period was calculated.

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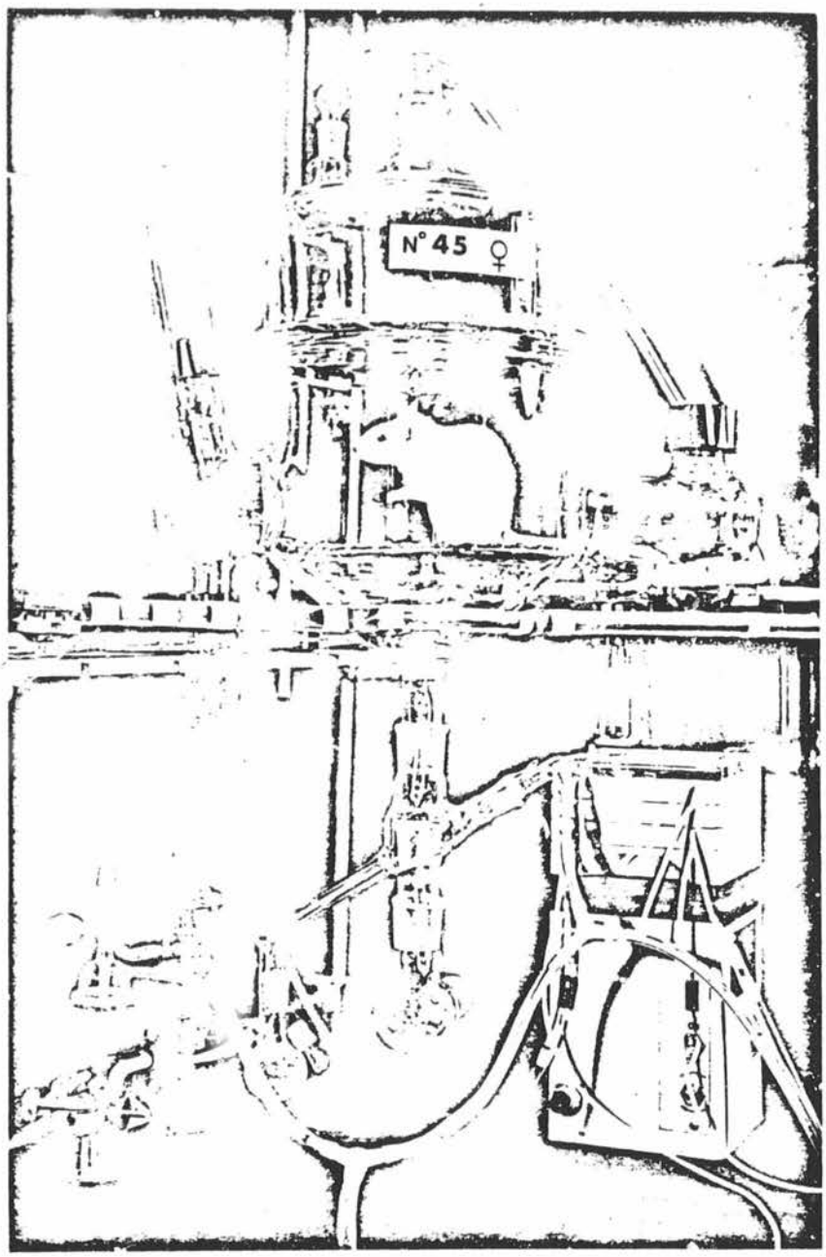

FIGURE 3 : Cage used for metabolic studies of TDI. The urines and faeces are collected separately in flasks located below the cage. A fraction of the air breathed out is bubbled through two flasks (lower left) containing an alkaline solution of phenylethylamine.

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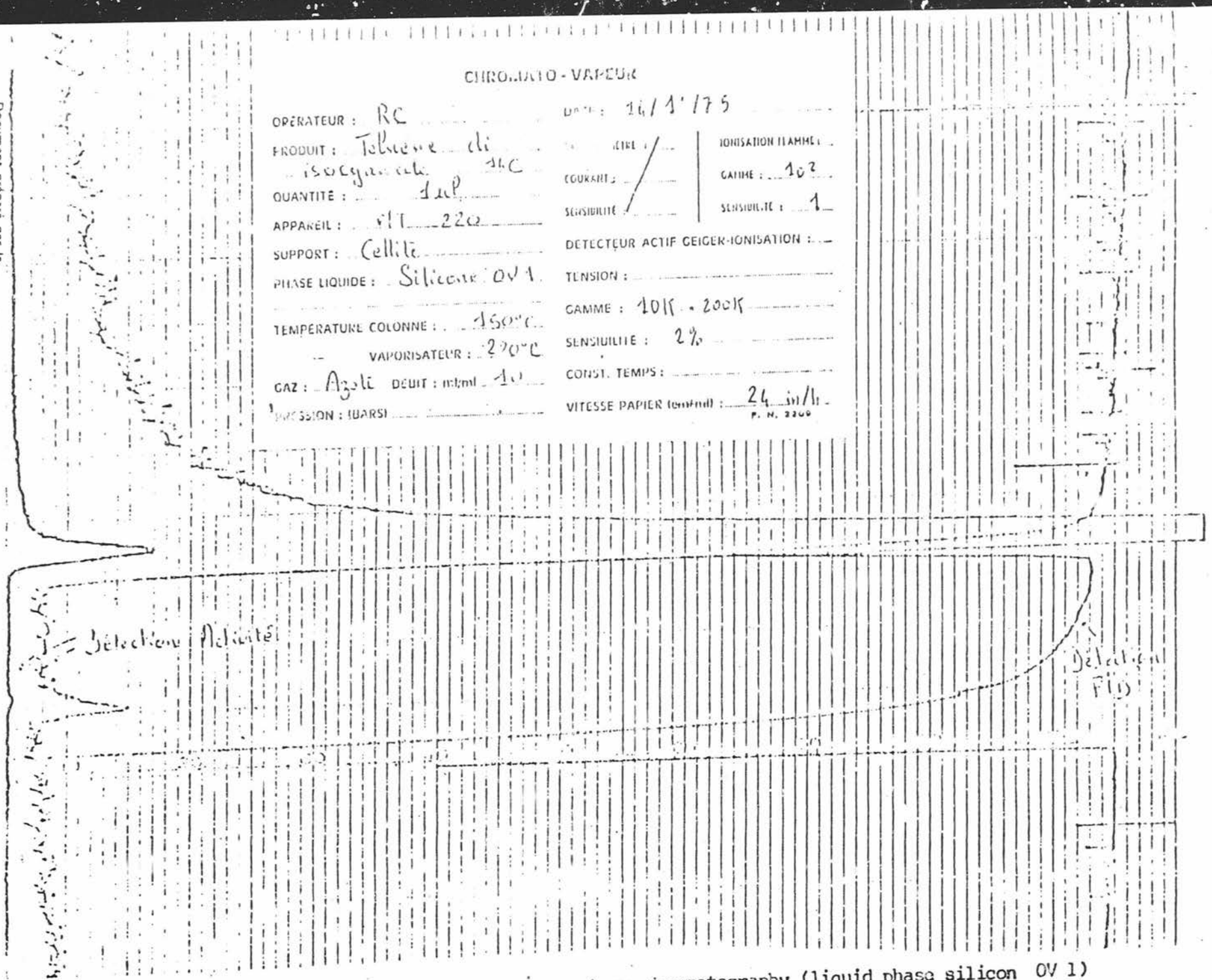

FIGURE 2 : Purity check of TDI. Recording obtained by gas phase chromatography (liquid phase silicon OV 1)

b) Purification and tests

The TDI was purified by sublimation. The radiochemical purity was checked by gas radiochromatography. Figures 1 and 2 show the radiochromatograms obtained after purification.

The final product contained 83.7 % of isomer 4 and 16.3 % of isomer 6. The specific radioactivity was 38 mCi/mM.

B - EQUIPMENT AND METHODS

a) Biological materials

The rats used in this investigation were of the Sprague Dawley strain (Charles River) weighing 150 to 200 g. The animals were kept fasting for 12 hours before and 2 hours after injection of TDI^{14}C. A single dose of TDI^{14}C was administered by intramuscular injection into the triceps of the right hind paw.

Five microliters of a solution containing 38 mCi/mM of TDI^{14}C in benzene was injected to each animal. An a posteriori check of total injected radioactivity was performed by adding the total excreted radioactivity to the radioactivity remaining in each animal when killed

b) Radioactivity measurement by liquid scintillation

The radioactivity from ^{14}C was measured by means of an Intertechnique (SL 32 PR) liquid scintillation spectrometer. The samples were prepared by mineralization (Intertechnique IN 4I0I unit). The count results were corrected for quenching and as a function of the specific efficiency of the counting instrument.

The scintillating solution has the following composition.

Toluene	400 ml
Phenylethylamine	330 ml
Methyl alcohol	220 ml
PPO	7 g
bis MSB	0.4 g
Water	20 ml

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No.	4	8	9
sex	♂	♀	♂
weight in g	164	159	172
TDI dose administered µg/animal	21.09	27.83	27.84

Table II : Individual doses of TDI administered to animals serving for an investigation of blood kinetics

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TABLE I : Individual results showing the evolution of ^{14}C radioactivity in the blood of rats as a function of time (see table II) after intramuscular injection of TDI ^{14}C. The results are expressed as a function of the radioactivity injected into the rats, measured per gram of blood (‰/g). The mean for each period was calculated.

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- Elimination by the respiratory tract

During the period in which the quantity of $^{14}CO_2$ breathed out by the rats was measured as a function of time, the cages were ventilated with an airflow of 300 ml/min. At the cage exit, a variable delivery pump was used to take 10 % of the total airflow for bubbling through two bottles in series containing an alkaline solution (phenylethylamine) (figure 3) which fixed the CO_2.

- At the end of the experiment, the animals were killed. The body of each animal was homogenized in the presence of a quantity of water equal to its weight. The radioactivity of the homogenate was measured.

RESULTS

The results presented below refer to a small number of animals. While they provide an understanding of the evolution and the magnitude of the phenomena observed, more experiments of this type are required to obtain accurate results.

a) Evolution of radioactivity in blood after intramuscular injection of TDI ^{14}C.

Table I gives the individual results of changes in blood radioactivity in rats having received a single intramuscular injection of TDI ^{14}C. The curves in Figures 4 and 5 illustrate the results of this table. In figure 4, the amount of radioactivity in the blood are plotted as a function of time in linear coordinates. A radioactivity peak located at about 24 hours can be observed. The curve in figure 5, for which the logarithm of blood concentrations is plotted as a function of time, can be broken down as follows. The first portion of the curve, which corresponds to increased blood radioactivity, enables evaluation of the diffusion rate of ^{14}C from muscle. After passing through a maximum, the second portion corresponds to the elimination of TDI ^{14}C and its metabolites from the blood. Although these results are not very accurate, this curve shows that the elimination follows a second order kinetic. Two hypotheses can be advanced to explain this result.

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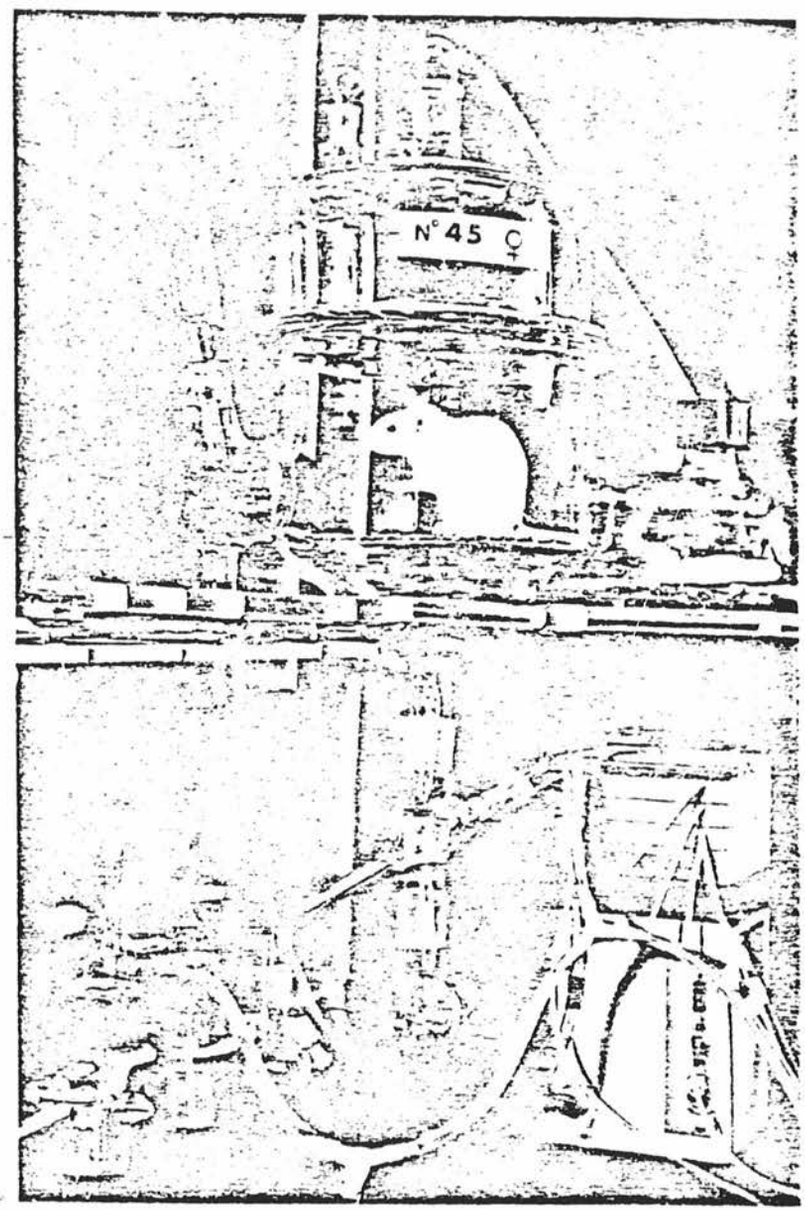

FIGURE 3 : Cage used for metabolic studies of TDI. The urines and faeces are collected separately in flasks located below the cage. A fraction of the air breathed out is bubbled through two flasks (lower left) containing an alkaline solution of phenylethylamine.

c) Evaluation of results

The results reported in this study correspond to measurements of the total radioactivity due to ^{14}C in the samples. They are expressed in each case as a fraction of the total radioactivity injected into the animal. The tables listing the means also show the standard error of these means, as well as the number of experiments used for calculating the mean.

C - EXPERIMENTAL PROCEDURES

All the experiments presented below were performed after giving a single dose of TDI ^{14}C to the animals.

a) Evolution of radioactivity in the blood stream

The single dose of TDI was injected to fasting rats. At regular time intervals, blood was taken under slight anesthesia from the cavernous sinus. These blood samples were immediately transferred and weighed in cupels employed for the preparation of liquid scintillation samples.

b) Excretion balance of radioactivity

In order to determine the excretion balances of TDI and its metabolic derivatives, the animals were kept individually in glass metabolism cages (Figure 3) designed to obtain separate collection of the urine and faeces, and collection of an aliquot of the CO_2 in the expired air. The animals were acclimatized to the cages for 48 hours before the start of the experiment. Aside from the 12 hours before and 3 hours after administration of the test compound, the animals received water and powdered food.

- The urines and faeces were collected during the periods of 0-12, 12-24, 24-48, 48-72, 72-96 and 96-120 hours after administration of the TDI ^{14}C, and the samples corresponding to each period were stored in the freezer while awaiting treatment. 200 microliters of urine were directly mineralized in the IN 4IOI unit. The faeces were oven-dried to constant weight, reduced to powder, and an aliquot of each sample was mineralized in the presence of 10 microliters of isobutyl alcohol. The latter was employed to facilitate combustion of the sample.

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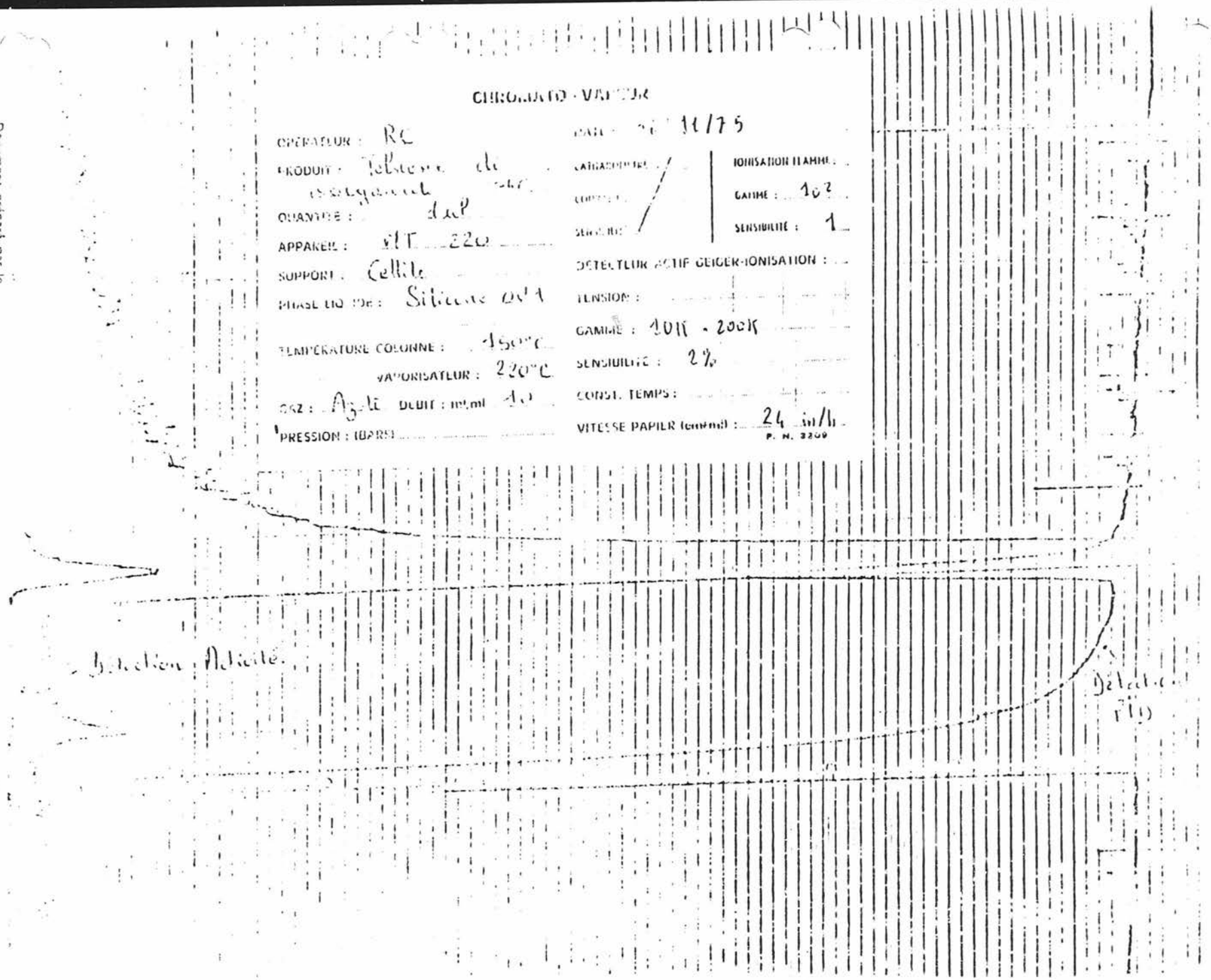

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FIGURE : Purity check of TDI. Recording obtained by gas phase chromatography (liquid phase silicon OV 1)

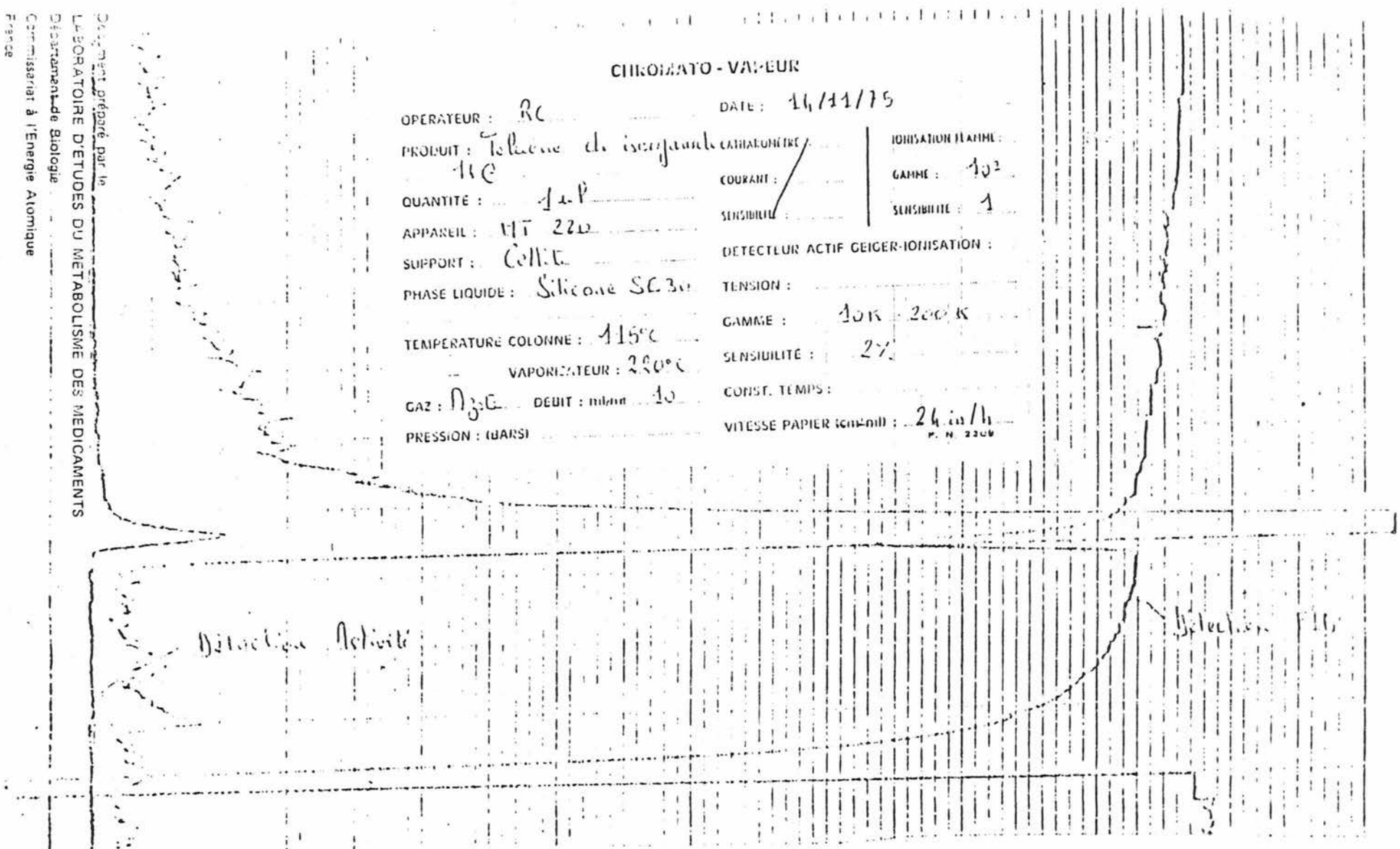

FIGURE 1 : Chemical purity check of TDI. Recording obtained by gas phase chromatography (liquid phase silicon SE30).

b) Purification and tests

The TDI was purified by sublimation. The radiochemical purity was checked by gas radiochromatography. Figures 1 and 2 show the radiochromatograms obtained after purification.

The final product contained 83.7 % of isomer 4 and 16.3 % of isomer 6. The specific radioactivity was 38 mCi/mM.

B - EQUIPMENT AND METHODS

a) Biological materials

The rats used in this investigation were of the Sprague Dawley strain (Charles River) weighing 150 to 200 g. The animals were kept fasting for 12 hours before and 2 hours after injection of TDI ^{14}C. A single dose of TDI ^{14}C was administered by intramuscular injection into the triceps of the right hind paw.

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b) Radioactivity measurement by liquid scintillation

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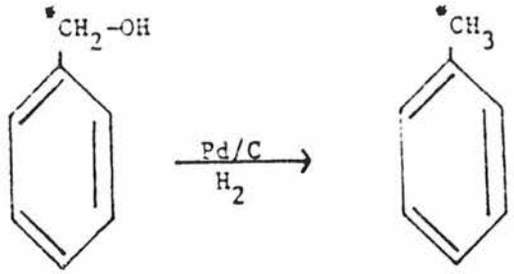
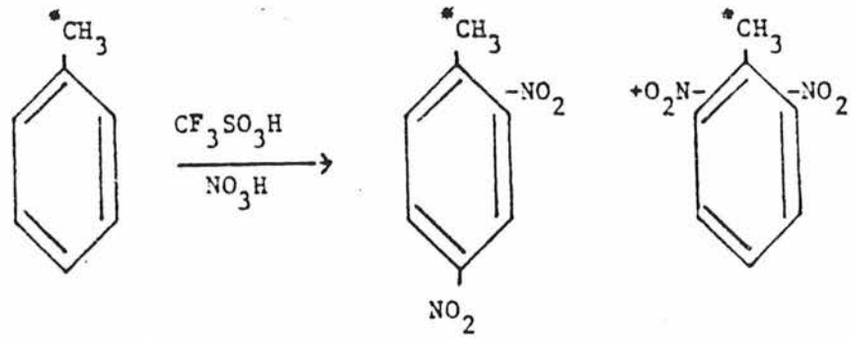
The scintillating solution has the following composition.

Toluene	400 ml
Phenylethylamine	330 ml
Methyl alcohol	220 ml
PPO	7 g
bis MSB	0.4 g
Water	20 ml

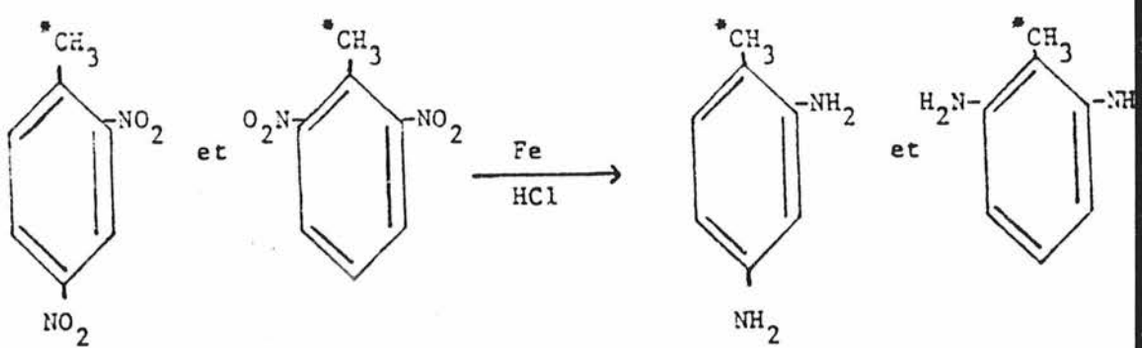
1) Reduction of benzyl alcohol to toluene :



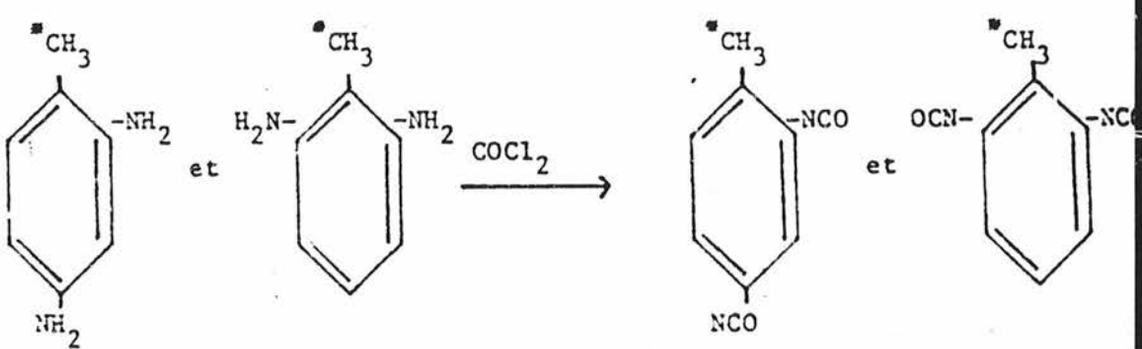
2) Nitration of toluene :



3) Reduction of dinitrotoluene :



4) Action of phosgene on the diamine :



Isomer 2-4 Isomer 2-6

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DEPARTEMENT DE BIOLOGIE
CEN-SACLAY — B.P. 2 — 91190 GIF sur YVETTE

10064

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LABORATOIRE D'ÉTUDES DU MÉTABOLISME DES MÉDICAMENTS

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E A 1

A STUDY OF THE DIFFUSION RATE OF TDI IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM

PRELIMINARY STUDY

Received at Safety
Committee Meeting,
13 July 1976

A STUDY OF THE DIFFUSION RATE OF TDI IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM

PRELIMINARY STUDY

The purpose of this work is to investigate the diffusion rate of TDI in the organism in rats contaminated via the respiratory tract. The determination of metabolic transformations undergone by this compound in contact with tissues and mucous membranes in animals will complete this investigation.

Before undertaking contamination by the respiratory tract and establishing the experimental procedure concerning this contamination, for technical reasons, a preliminary study was conducted on rats, in order to estimate the diffusion rate of TDI in blood, after intramuscular injection. The urinary and fecal elimination rates were also investigated.

TDI ^{14}C was used in this investigation.

A - SYNTHESIS OF THE MOLECULE

The TDI ^{14}C was synthesized by the labelled Molecule Laboratory of the Commissariat à l'Energie Atomique at Saclay (Mr. Pichat).

a) Synthesis

The following scheme was followed for synthesis of the labelled compound :

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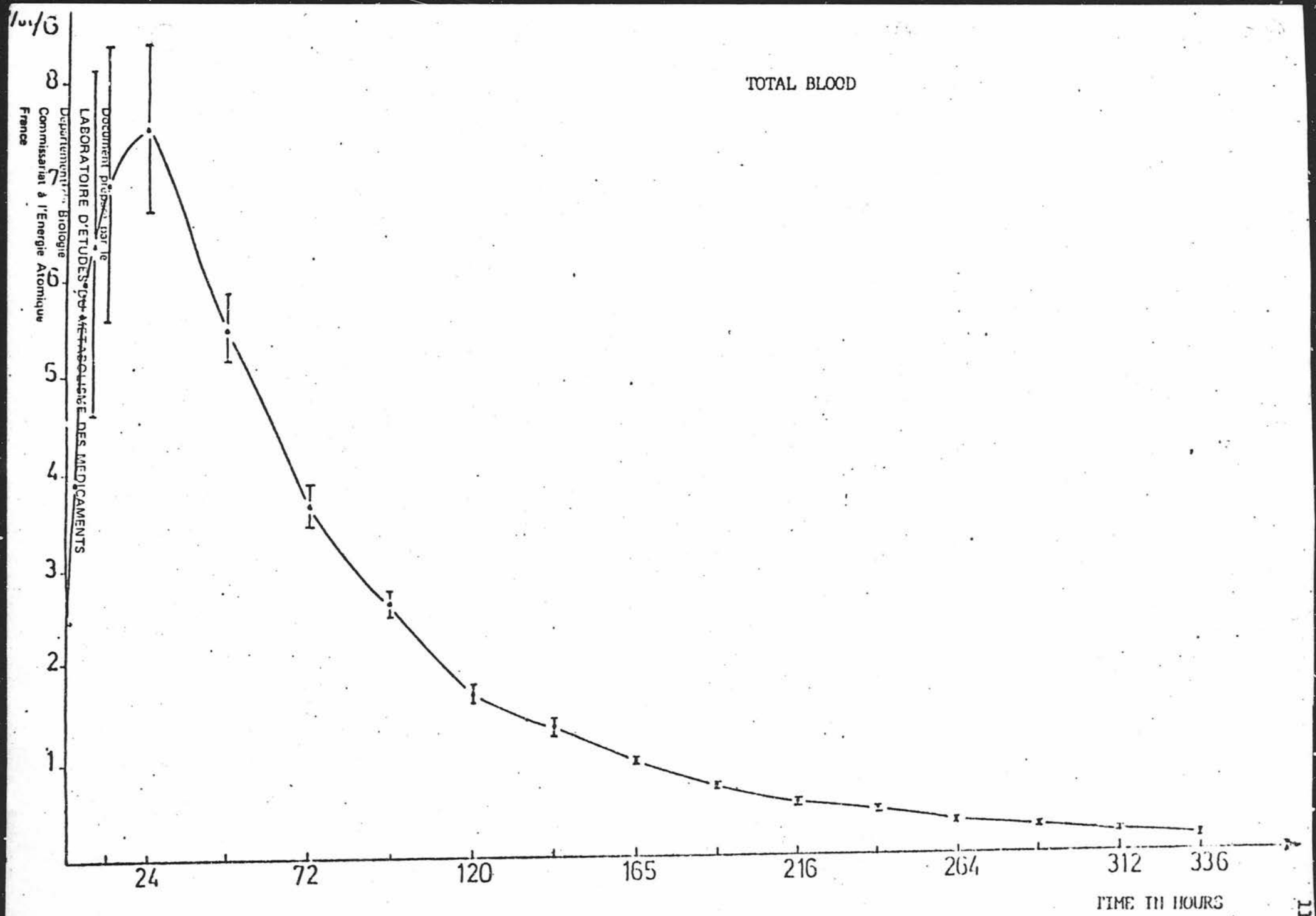

FIGURE 4 : Variation as a function of time of the concentration of ^{14}C radioactivity in the blood of rats having

- a ^{14}C compound ($TDI^{14}C$ or one metabolite) is distributed in two compartments in the organism,
- we are in the presence of two labelled derivatives, metabolites with different elimination kinetic parameters.

Graphic interpretations and calculations make it possible to estimate the following parameters :

- for elimination : the relative magnitudes of the two components of the elimination curve are in a ratio of about 3.8.

The half-lives of the two components are 32 hours for the larger compartment and 73 hours for the smaller.

- for diffusion : the T 1/2 of diffusion from the muscle of injected $TDI^{14}C$ is about 30 minutes.

b) Urinary and fecal excretion of radioactivity after intramuscular injection of a single dose of $TDI^{14}C$.

Tables III and IV give the individual results of fractions of radioactivity eliminated during each sampling period. Tables V and VI, which resume these results, make it possible to follow the progress of cumulative quantities of radioactivity deriving from $TDI^{14}C$ or its labelled derivatives as a function of time. A check revealed that the elimination of ^{14}C in the form of $^{14}CO_2$ through the respiratory tract is negligible, showing that, in rats, there is no transformation of the molecule by demethylation of the radical $-^{14}CH_3$.

The curves in figures 6 and 7 illustrate the results given in the tables. To summarize, these experiments show the following :

- that the urinary elimination of $TDI^{14}C$ and its metabolites labelled with ^{14}C is greater than their fecal elimination (53 % as compared with 39 %),
- that the excreta recovery balance is better than 92 %. Four per cent is found in the carcases after the animals are killed.

CONCLUSION

The purpose of these experiments was to evaluate the diffusion rate

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Rat n° / Sex / TIME (h)	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
FAECES					
0 - 6	0	3. 64	0	0	0. 91 ± 0. 45
6 - 12	26. 56	18. 45	9. 23	23. 93	19. 54 ± 1. 92
12 - 24	54. 09	33. 09	18. 25	45. 99	37. 85 ± 3. 92
24 - 48	150. 19	94. 26	215. 08	109. 15	142. 17 ± 13. 51
48 - 72	58. 49	66. 75	117. 59	59. 99	75. 70 ± 7. 04
72 - 96	34. 07	38. 38	43. 54	23. 32	34. 83 ± 2. 15
96 - 120	17. 41	20. 75	14. 21	15. 79	17. 04 ± 0. 70
120 - 144	11. 35	15. 57	7. 99	12. 61	11. 88 ± 0. 78
144 - 168	6. 58	11. 99	7. 02	11. 41	9. 25 ± 0. 71
168 - 192	6. 65	11. 32	8. 71	11. 71	9. 60 ± 0. 59
192 - 216	6. 26	7. 74	4. 12	13. 04	7. 79 ± 0. 95
216 - 240	4. 76	10. 67	3. 40	12. 90	7. 93 ± 1. 14
240 - 264	3. 39	8. 48	3. 58	11. 07	6. 63 ± 0. 95
264 - 288	3. 69	4. 89	2. 84	6. 07	4. 37 ± 0. 35
288 - 312	1. 83	4. 21	0. 58	5. 46	3. 02 ± 0. 55
312 - 336	1. 77	2. 44	1. 49	3. 19	2. 22 ± 0. 19
336 - 360	1. 99	1. 65	1. 56	1. 36	1. 64 ± 0. 07

TABLE IV : Individual results showing fecal elimination of ^{14}C after intramuscular injection of a single dose of TDI ^{14}C in rats (see table VII). The results are expressed as a fraction of the dose injected (°/oo). The mean for each period was calculated.

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Rat n° / Sex / TIME (h)	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
FAECES					
0 - 6	0	3.64	0	0	0.91 ± 0.45
6 - 12	26.56	22.09	9.23	23.93	20.45 ± 1.93
12 - 24	80.66	55.18	27.48	69.92	58.31 ± 5.76
24 - 48	230.84	149.43	242.56	179.07	200.47 ± 10.95
48 - 72	289.33	216.18	360.15	239.05	276.17 ± 15.94
72 - 96	323.41	254.56	403.69	262.38	311.01 ± 17.26
96 - 120	340.82	275.30	417.90	278.17	328.05 ± 16.77
120 - 144	352.17	290.88	425.90	290.78	339.93 ± 16.04
144 - 168	358.75	302.87	432.92	302.19	349.18 ± 15.45
168 - 192	365.40	314.18	441.63	313.90	358.77 ± 15.08
192 - 216	371.66	321.92	445.76	326.95	366.57 ± 14.33
216 - 240	376.42	332.59	449.16	339.85	374.51 ± 13.33
240 - 264	379.80	341.07	452.74	350.92	381.13 ± 12.62
264 - 288	383.49	345.96	455.57	356.99	385.50 ± 12.32
288 - 312	381.64	350.17	456.15	362.45	387.60 ± 11.87
312 - 336	383.40	352.61	457.64	365.64	389.82 ± 11.73
336 - 360	385.39	354.26	459.20	367.00	391.46 ± 11.73
TOTAL URINE + FAECES	950.52	943.83	885.97	911.80	923.03 ± 7.48

TABLE VI : Individual results showing the cumulative fecal elimination of ^{14}C as a function of time, after administration of a single dose of TDI ^{14}C by intramuscular injection in rats (see table VII). The results are expressed in parts per 1000 of the dose injected. The mean for each period as well as the overall balance are calculated.

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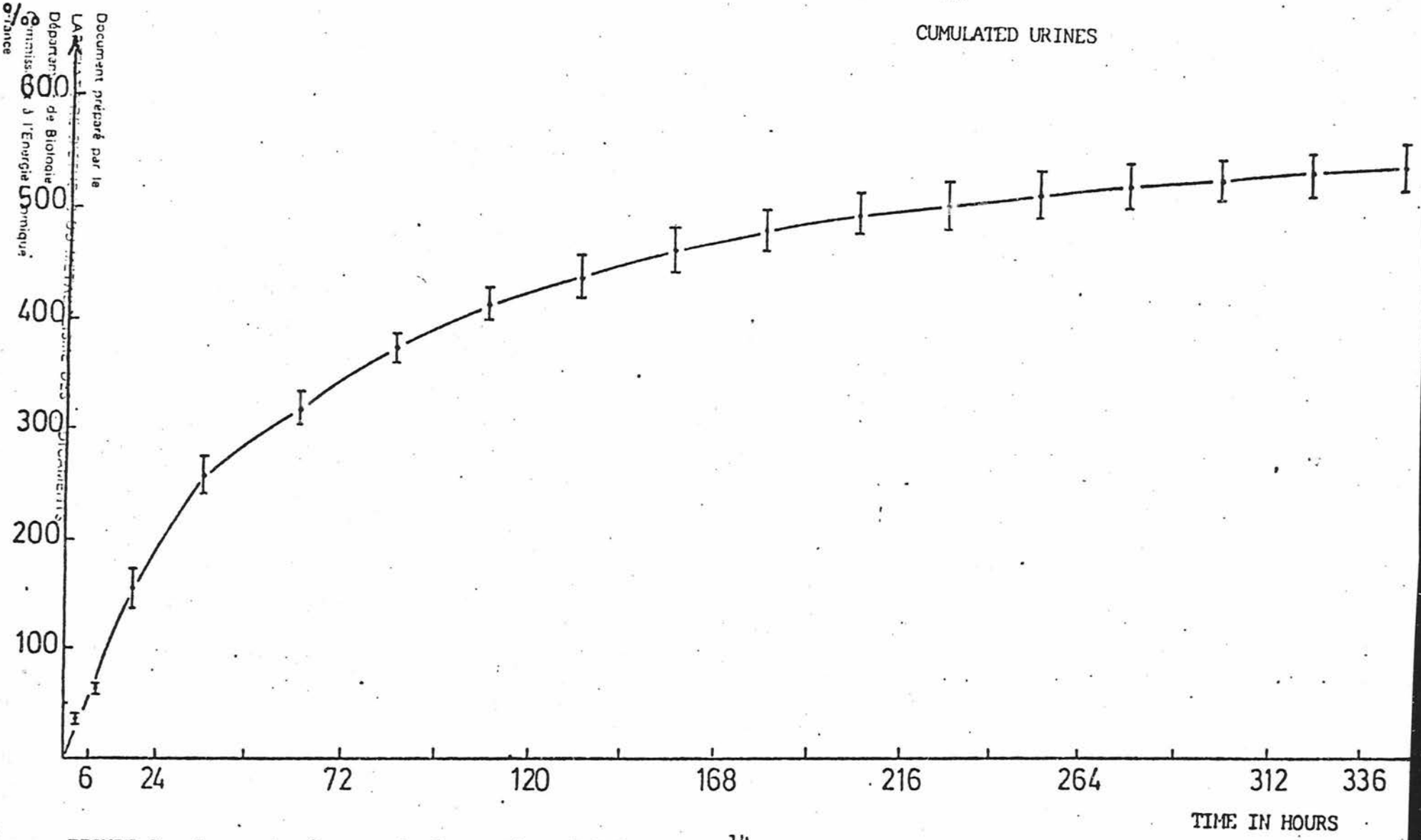

FIGURE 6 : Curve showing cumulative radioactivities of TDI^{14}C and its labelled metabolites in urine as a function of t after intramuscular injection of a single dose of TDI^{14}C. This curve was plotted from the results given in table V.

of TDI^{14}C from the muscle, before considering contamination of rats via the respiratory tract. This diffusion rate is fairly rapid with a T 1/2 of 30 minutes. Our experience suggests that diffusion from the respiratory tract should be even more rapid. Moreover, the relatively slow elimination of derivatives of TDI^{14}C labelled with ^{14}C should permit observation of blood, urinary and fecal radioactivities with the new experimental procedure.

The autoradiograph of the entire animal (see Appendix) shows that TDI^{14}C and its labelled metabolites are practically diffused throughout the organs, with a high concentration in the excretion organs.

From the purely kinetic standpoint, the distribution curves based on distribution in blood and on the excretion balance suggest that in addition to TDI, metabolites are formed :

- one or two important metabolites diffusible through the majority of organs :
  - with relatively slow elimination rates,
  - with an elimination balance approaching 100 % in 15 days.

These hypotheses will only be corroborated by additional investigations

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A STUDY OF THE DIFFUSION RATE OF TDI IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM

PRELIMINARY STUDY

The purpose of this work is to investigate the diffusion rate of TDI in the organism in rats contaminated via the respiratory tract. The determination of metabolic transformations undergone by this compound in contact with tissues and mucous membranes in animals will complete this investigation.

Before undertaking contamination by the respiratory tract and establishing the experimental procedure concerning this contamination, for technical reasons, a preliminary study was conducted on rats, in order to estimate the diffusion rate of TDI in blood, after intramuscular injection. The urinary and fecal elimination rates were also investigated.

TDI ^{14}C was used in this investigation.

A - SYNTHESIS OF THE MOLECULE

The TDI ^{14}C was synthesized by the labelled Molecule Laboratory of the Commissariat à l'Energie Atomique at Saclay (Mr. Pichat).

a) Synthesis

The following scheme was followed for synthesis of the labelled compound :

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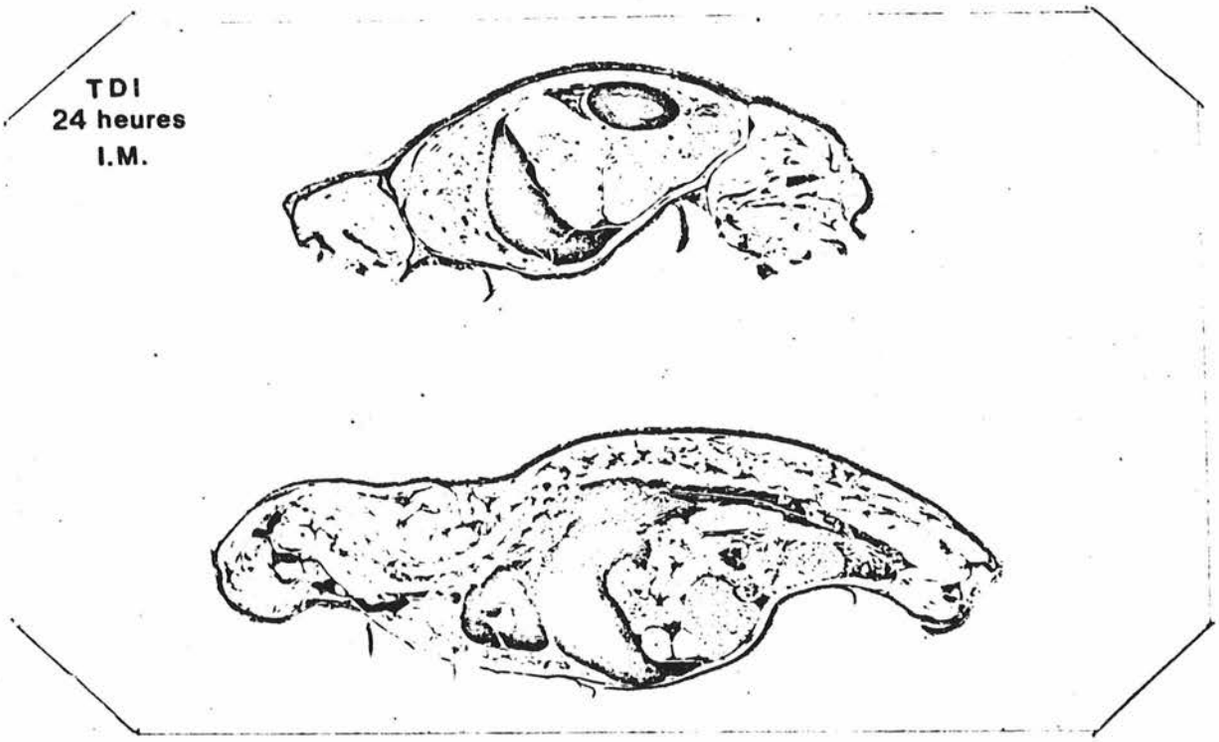

PLATE I

Table I

time in hours / organ	24 ♀
kidneys: medullar zone	++++
kidneys: cortical zone	++
liver	++
large intestine	++
spleen	++
heart	++
salivary glands	++
skin	++
lungs	+
bone marrow	+
bladder	

Quantity of radioactivity localized by autoradiography in the rat.

++++ very strong

+++ strong

++ medium

+ weak

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APPENDIX I

AUTORADIOGRAPHY OF THE ENTIRE ANIMAL

PROCEDURE : A rat weighing 100 g receives an intramuscular injection of TDI^{14}C (18 µCi). Twenty four hours after administration of the chemical, the animal is killed and rapidly frozen by immersion in liquid nitrogen. The frozen rat is stored for 24 hours in a freezer at -25°C before the preparation of sections. Ullberg's technique (1954) was employed for preparing the sections. A leitz microtome was used with a plate cooled to -30°C.

50 µ sections parallel to the sagittal axis of the animal were prepared. Collected on adhesive tape, they were dehydrated and then placed against a "Kodirex" monolayer (Kodak) radiological film. The films were developed after five days of exposure. The black areas enable localization of the radioactivity due to ^{14}C introduced into the body of the animal in the form of TDI^{14}C.

RESULTS

Plate 1 shows the autoradiographs of a rat killed 24 hours after administration of the labelled medicament. This period corresponds approximately to the activity peak in the plasma of the animal.

The relative importance of tissue fixation of the chemical is shown in table I. These patterns were obtained from preparations of the same thickness (50 µ). The exposure period was seven days in all cases.

REFERENCE

Ullberg S., 1954, Acta Radiol. Supp. 118

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A study of the diffusion of MDI in rats contaminated via the respiratory system.

ATTACHMENT TO REPORTS SC/EA-13 & 14

Reports SC/EA-13 & 14.

Dr. M.Sumi, Chairman of Toxicological Sub-Committee (Far East Region) has sent to the Safety Office the following comment :

Dear Dr. Wilson,

We read through two reports of preliminary studies on the diffusion of TDI and MDI in rats. We have no comments on them for the moment, since they are preliminary ones and especially detailed discussions are not given with regard to graphic interpretations and calculations. It should only be added that I don't know whether the term "second order kinetic" is suitable or not in the case of the elimination of TDI (p.8).

Attached to 10665

EA 4 - METABOLISM 1976 - Visit to Saclay on Friday 9th, 1976

- Mr. Istin has not yet recieved the contract from Mr. Cunningham.* He is a bit worry not to have official paper in hand about the 1976 work and he needs it to send the invoice and to receive 50%.

- Neverthe less the attempts are on stream on TDI and the first results give a lot of information :

- Just after inhalation of 1 ppm during 1 hour, as asked by Dr. Loeser, Mr. Istin killed several rats to determine the radioactivity level in 16 organs plus blood and plasma.
- The diffusion rate is rapid in the whole body as forscen by the preliminary attempts.
- The kiduney is the most concerned and more than liver
- Surprisingly , the stomac seems to be more concerned than the liver
- The rats have very well supported and the lungs were intact but it is necessary to wait for the results after histological study
- The sensitivity is very confortable. The concentration or the time could be divided easily by 10 may be more.
- The ratio $\frac{\text{Radioactivity of urine}}{\text{Radioactivity of feces}} = \frac{30}{70}$

  it is a surprising to see that is the reverse of that in the preliminary test by injection
- The first autoradio chromatographies are very rich and fascinating. Just after the intoxication three very polar products, different from TDI, are appearing. These products are very rapidly eliminated. But after that, several non polar products are getting visible and fading slowly.

Mr. Istin would like to meet Dr. Loeser to comment these first results and suggests a date before the end of the month. I said that it could be possible (on 28/7) if Dr. Loeser is not yet in holiday.

He did not forget that he had invited Dr. Brochhagen and Dr. Loeser to participate to the inhalation procedure but he was not allowed because security regulation in C.E.A

.../

* But we have recieved copy on June 18th.

Mademoiselle BODIN from Eurane helped for training (without radioactivity) for the control of the TDI in the atmosphere with the osmopile and met no problem.

Labelled foams

Mr. Istin informed me that the preparation of the 4 labeled foams and polyurée was estimated to about 2.300 to 2.500 $. He will send me an invoice for that.

Stability of labelled TDI and MDI

No variation has been formed regarding the purity verses time.

June 12 th, 1976

12/7/1976

APPENDIX I

Quantities Injected	T D I 5 µl of 38 mCi/mM benzene solution	M D I 8 µl of 24,7 m Ci/mM benzene solution
BLOOD		
maximum	24 h	24 h
half time diffusion	0 h 30	78 h
half time elimination	32 h (second order) 73 h 2 metabolites	12 h (first order)
Excretion balance		
balance recovery (urine + feces)	92 %	25%
in the killed animal	4 %	# 70 %
air expired	0	0 *
Elimination balance	100% in 15 days	very long
Elimination urine / faces	53 % / 39 %	1 / 3,6

* by sintillation was some radioactivity appearing after 2 days but by ionisation there is no C 14 as for TDI

SUMMARY OF THE RESULTS OBTAINED BY THE PRELIMINARY ATTEMPTS OF METABOLISME OF TDI and MDI

(Reports of C.E.A - Departement de Biologie)

The main aim of this preliminary study was to have on idea of the diffusion rate of TDI and MDI within rats contamine ted via respiratory system.

The first step was to prepare labelled TDI and MDI with C 14 on the methyl-group for TDI and on the methylen group for MDI. The radio chemical purity was checked by gas radiochromatography.

The second step was to investigate after intramuscular injection in order to estimate the diffusion rate in the blood, urine and feces.

The results reported correspond to maesurements of the total radioactivity due to C 14 in the samples and are expressed in each case as a fraction of the total radioactivity injected into the animal.
Variation during 336 h were recorded. At the end, the animals were killed and the radioactivity of the whole body was maesured.
The first part of the curve in logarithmic coordinate gives the rate of diffusion from muscle. The second part of the curve, after the maximum, gives the rate of elimination of the metabolites.
The results are grouped in the tabel (Appendix I)

This preliminary work was helpful to reach accuracy and savety in the further investigation by inhalation.

A STUDY OF THE DIFFUSION OF MDI IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM

Saclay

PRELIMINARY STUDY

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A STUDY OF THE DIFFUSION OF MDI
IN RATS CONTAMINATED VIA THE RESPIRATORY SYSTEM

PRELIMINARY STUDY

The purpose of this work is to investigate the diffusion rate of MDI in the organism in rats contaminated via the respiratory tract. The determination of metabolic transformations undergone by this compound in contact with tissues and mucous membranes in animals will complete this investigatio

Before undertaking contamination by the respiratory tract and establishing the experimental procedure concerning this contamination, for technical reasons, a preliminary study was conducted on rats, in order to estimate the diffusion rate of MDI in blood, after intramuscular injection. The urinary and fecal elimination rates were also investigated.

MDI (^{14}C) was used in this investigation.

A - SYNTHESIS OF THE MOLECULE

The MDI (^{14}C) was synthetized by the labelled Molecule Laboratory of the Commissariat à l'Energie Atomique at Saclay (Mr. Pichat).

a) Synthesis

The following scheme was followed for synthesis of the labelled compour

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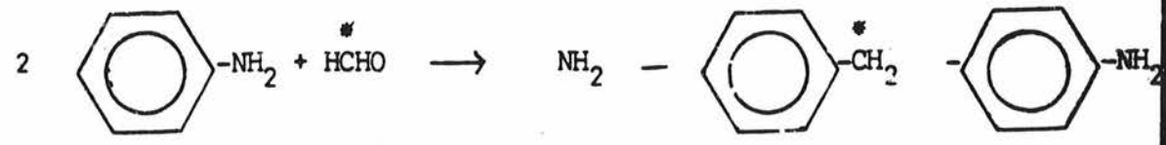
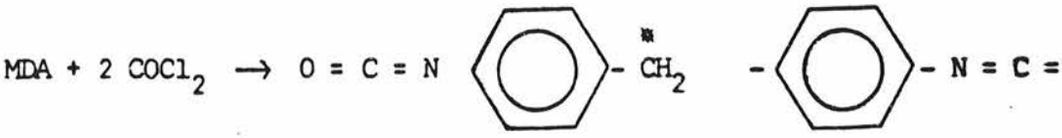
1) Formol condensation with aniline to give the MDA



2) To obtain MDI, raw MDA is treated by $COCl_2$



b) Purification and tests

MDA was purified by chromatography on a column of "Silice H". Thin layer chromatography and UV titration are used as tests of the purification (Fig. 1 and 2). This pure MDA is used to ended the synthesis of MDI. The results of radiochemical controls appear on figures 2 and 3

The final product has a radioactivity of 24,7 mCi/mM.

B - EQUIPMENT AND METHODS

a) Biological materials

The rats used in this investigation were of the Sprague Dawley strain (Charles River) weighing 150 to 200 g. The animals were kept fasting for 12 hours before and 2 hours after injection of MDI (^{14}C). A single dose of MDI (^{14}C) was administered by intramuscular injection into the triceps of the right hind paw.

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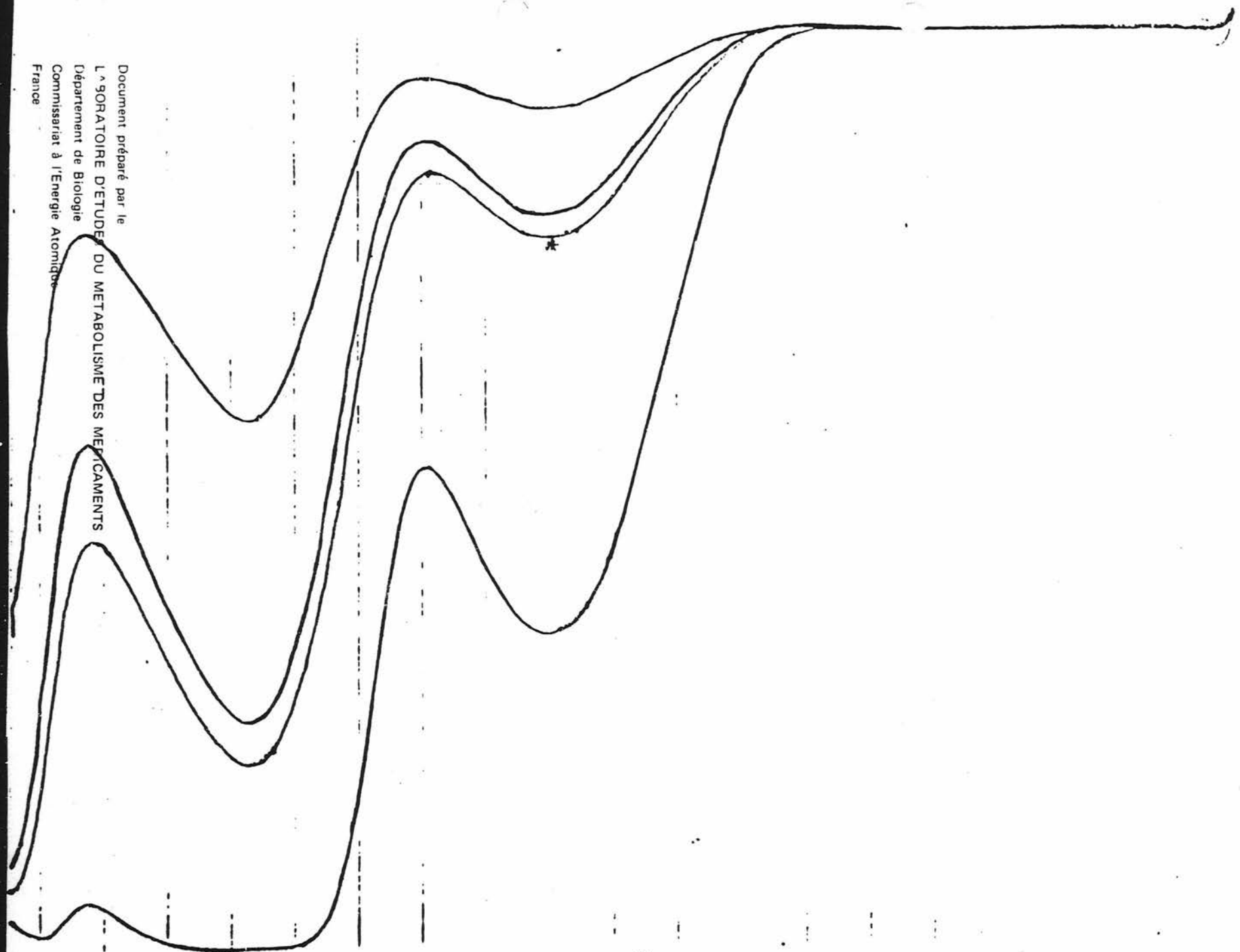

Figure 1 - Chemical identity check of MDA (^{14}C) UV spectrum

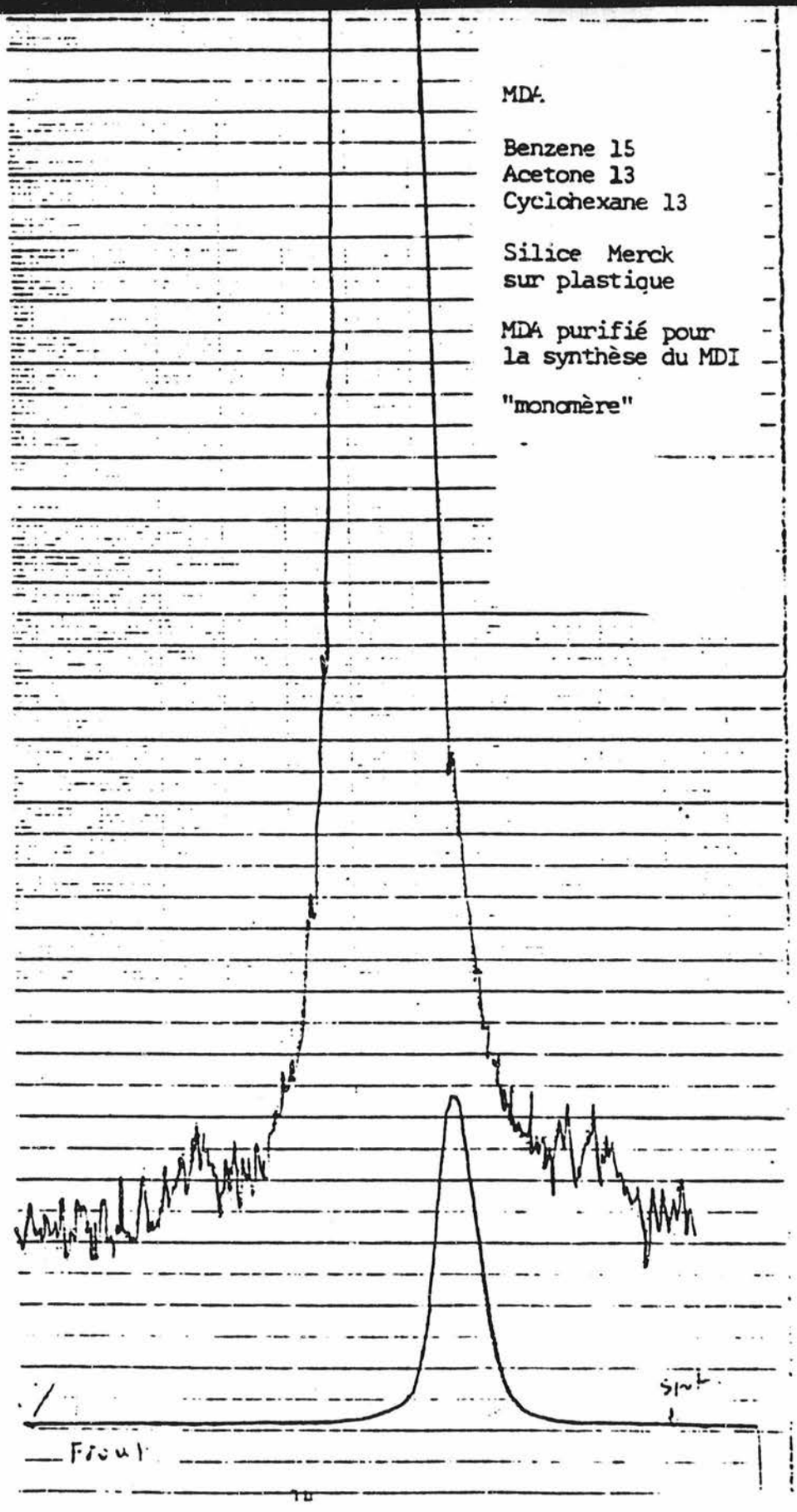

Figure 2 : Radio chemical control of MDA (^{14}C). The chromatography has been developp with the system of solvent : Benzene 15
Acetone 13
Cyclohexane 13

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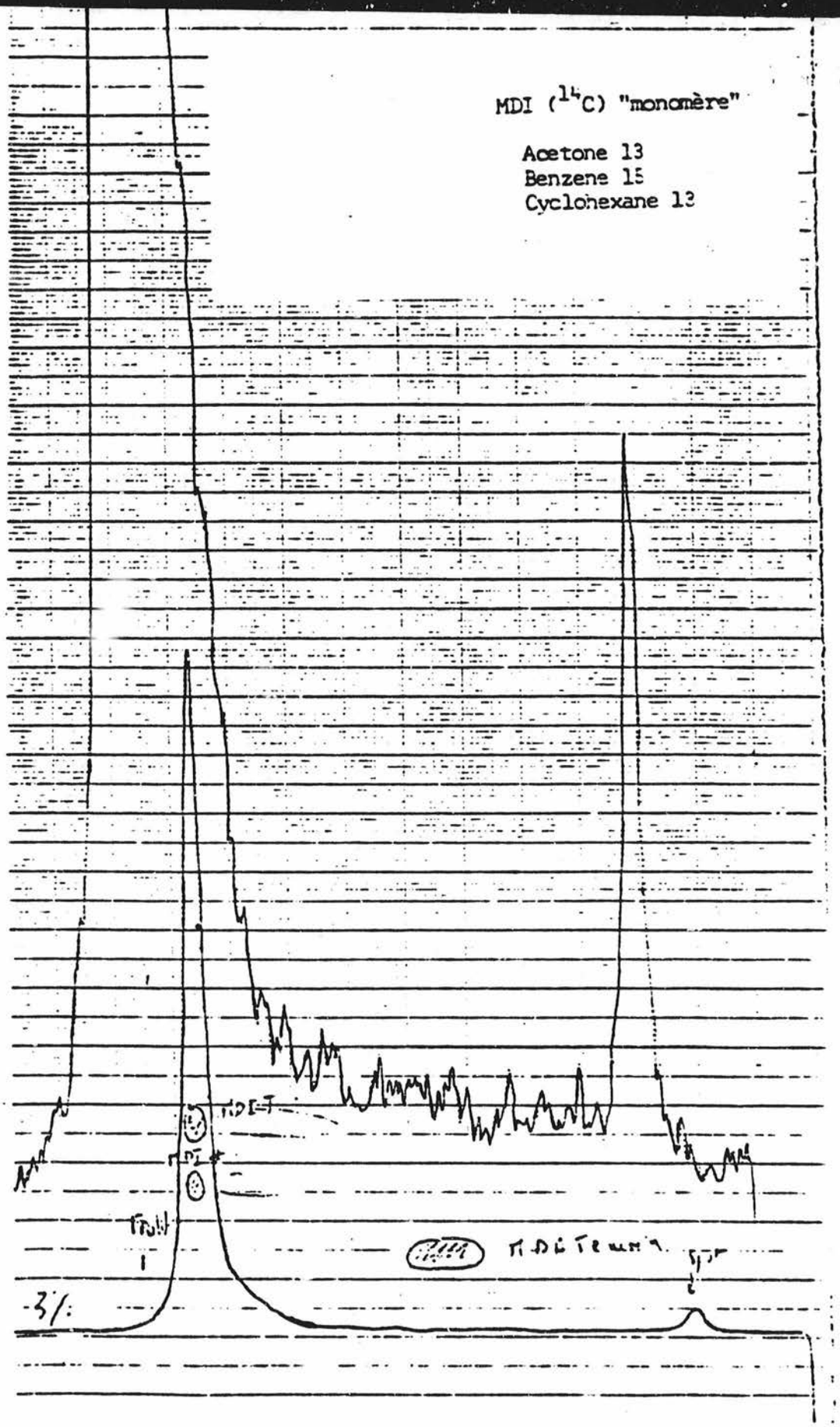

Figure 3 : Radio chemical control of MDI (^{14}C) "monomeride". The chromatography has been developp with the system of solvent : Benzene 15
Acetone 13
Cyclohexane 13

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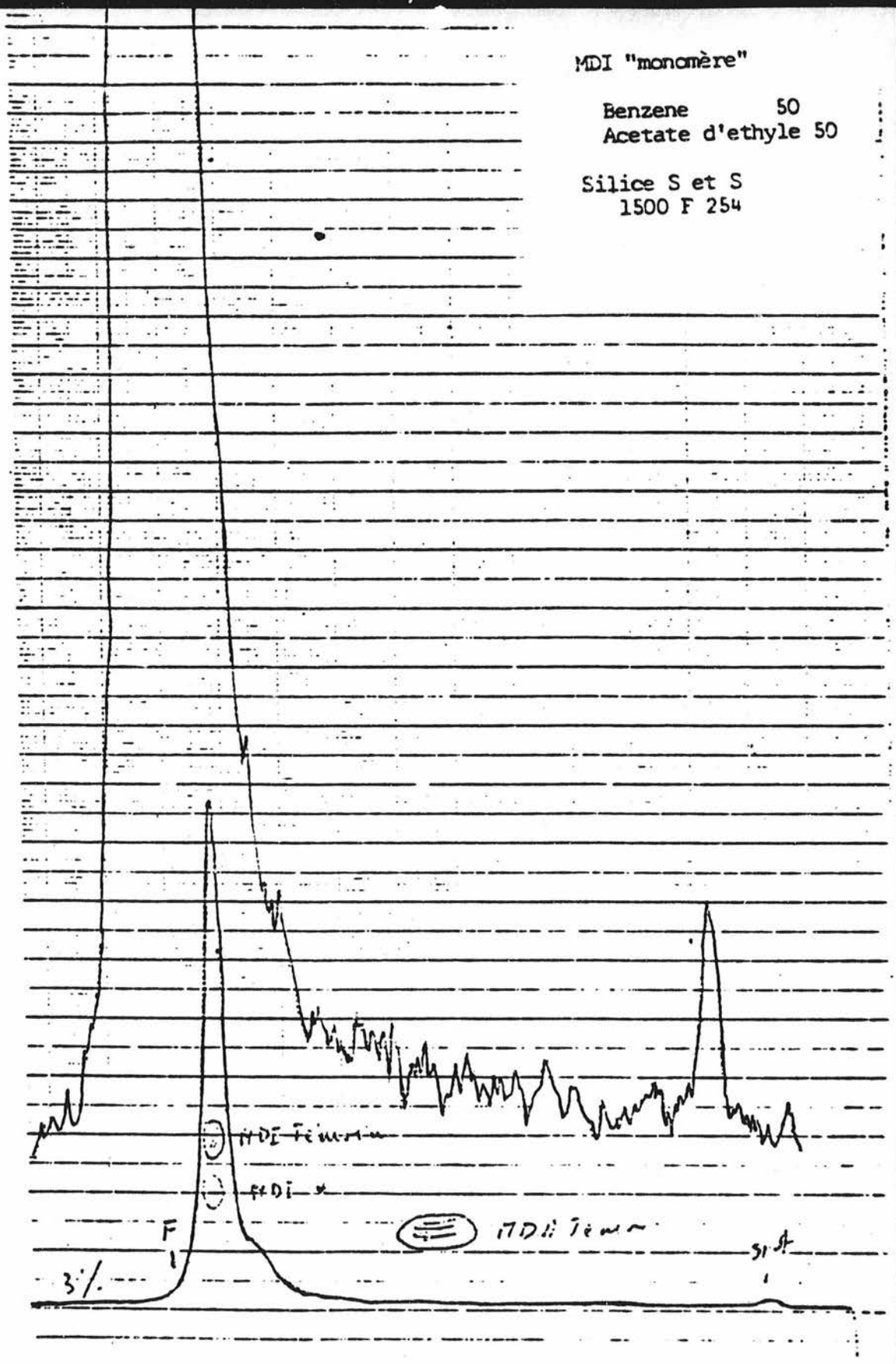

Figure 4 : Radio chemical control of MDI (^{14}C) "monomeride". The chromatography has been developp with the system of solvent : Benzene 50
Acetate d'ethyle 50

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Eight microliters of a solution containing 24,7 mCi/mM of MDI (^{14}C) in benzene was injected to each animal. An a posteriori check of total injected radioactivity was performed by adding the total excreted radioactivity to the radioactivity remaining in each animal when killed

b) Radioactivity measurement by liquid scintillation

The radioactivity from ^{14}C was measured by means of an Intertechnique (SL 32 PR) liquid scintillation spectrometer. The samples were prepared by mineralization (Intertechnique IN 4IOI unit). The count results were corrected for quenching and as a function of the specific efficiency of the counting instrument.

The scintillating solution has the following composition.

Toluene	400 ml
Phenylethylamine	330 ml
Methyl alcohol	220 ml
PPO	7 g
bis MSB	0.4 g
Water	20 ml

c) Evaluation of results

The results reported in this study correspond to measurements of the total radioactivity due to ^{14}C in the samples. They are expressed in each case as a fraction of the total radioactivity injected into the animal.

C - EXPERIMENTAL PROCEDURES

All the experiments presented below were performed after giving a single dose of MDI (^{14}C) to the animals.

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a) Evolution of radioactivity in the blood stream

The single dose of MDI was injected to fasting rats. At regular time intervals, blood was taken under slight anesthesia from the cavernous sinus. These blood samples were immediately transferred and weighed in cupels employed for the preparation of liquid scintillation samples

b) Excretion balance of radioactivity

In order to determine the excretion balances of MDI and its metabolic derivatives, the animals were kept individually in glass metabolism cages (Fig. 5) designed to obtain separate collection of the urine and faeces, and collection of an aliquot of the CO_2 in the expired air. The animals were acclimatized to the cages for 48 hours before the start of the experiment. Aside from the 12 hours before and 3 hours after administration of the test compound, the animals received water and powdered food.

- The urines and faeces were collected during the periods of 0-12, 12-24, 24-48, 48-72, 72-96 and 96-120 hours after administration of the MDI (^{14}C), and the samples corresponding to each period were stored in the freezer while awaiting treatment. 200 microliters of urine were directly mineralized in the IN 4IoI unit. The faeces were oven-dried to constant weight, reduced to powder, and an aliquot of each sample was mineralized in the presence of 10 microliters of isobutyl alcohol. The latter was employed to facilitate combustion of the sample.

- Elimination by the respiratory tract :

During the period in which the quantity of $^{14}CO_2$ breathed out by the rats was measured as a function of time, the cages were venti-

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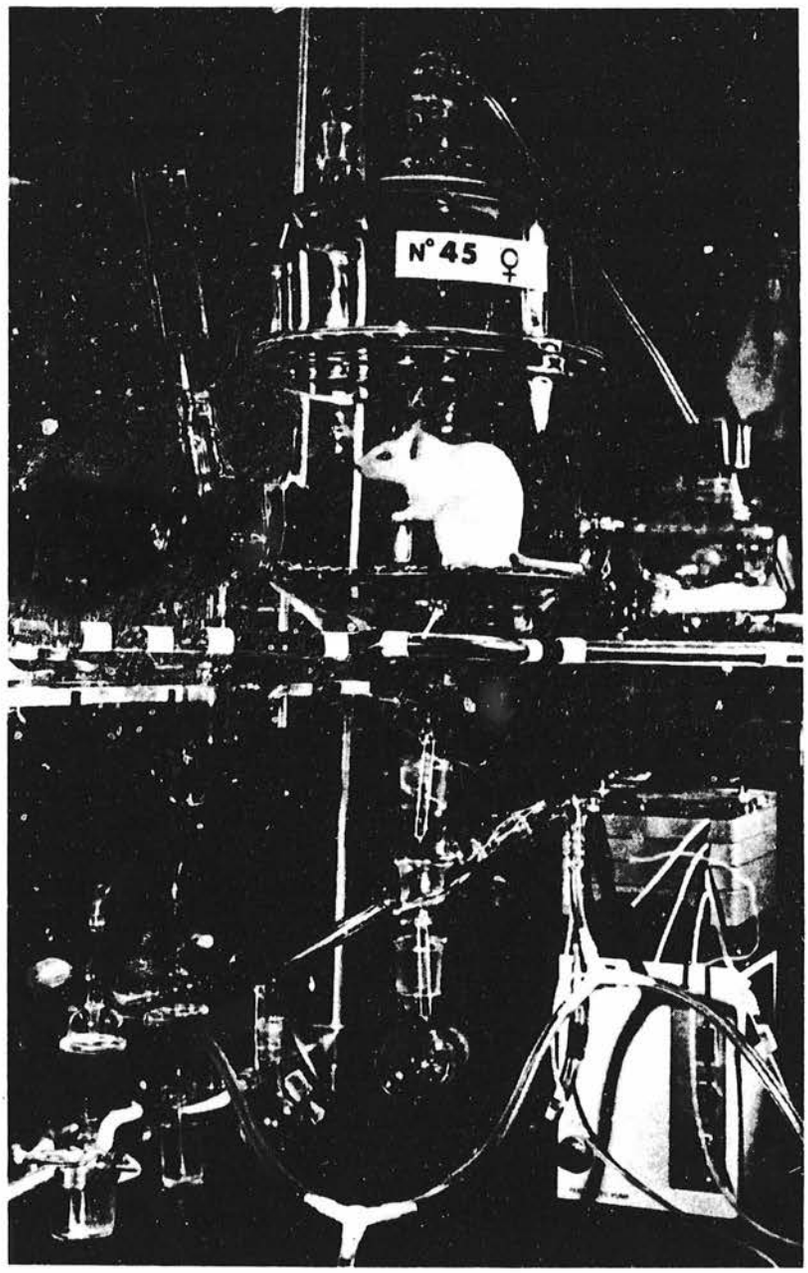

Figure 5 : Cage used for metabolic studies of MDI. The urines and faeces are collected separately in flasks located below the cage. A fraction of the air breathed out is bubbled through two flasks (lower left) containing an alkaline solution of phenylethylamine.

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lated with an airflow of 300 ml/min. At the cage exit, a variable delivery pump was used to take 10 % of the total airflow for bubbling through two bottles in series containing an alkaline solution (phenylethylamine) (Fig. 5) which fixed the CO_2.

- At the end of the experiment, the animals were killed. The body of each animal was homogenized in the presence of a quantity of water equal to its weight. The radioactivity of the homogenate was measured.

RESULTS

The results presented below refer to a small number of animals. While they provide an understanding of the evolution and the magnitude of the phenomena observed, more experiments of this type are required to obtain accurate results.

a) Evolution of radioactivity in blood after intramuscular injection of MDI (^{14}C).

Table I gives the individual results of changes in blood radioactivity in rats having received a single intramuscular injection of MDI (^{14}C). The curves in figures 6 and 7 illustrate the results of this table. In figure 6 the amount of radioactivity in the blood are plotted as a function of time in linear coordinates. A radioactivity peak located at about 24 hours can be observed. The curve in figure 7, for which the logarithm of blood concentrations is plotted as a function of time, can be broken down as follows.

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Time h \ N° / Sex	5 ♂	6 ♂	7 ♀	8 ♀	Mean
1	0,23	0.07	0.22	0,11	0,18
2	0.77	0,27	0.62	0,17	0.46
3	0.95	0,53	0.97	0,21	0,66
4	1,05	0.89	1.66	0.27	0.97
6	1.27	1.34	2,44	0,34	1.35
8	1,29	1,82	3.30	0,41	1,70
12	1,74	2,28	5.08	0.57	2,42
25	2,88	2.62	5,42	1,76	3,17
48	2,83	2.03	3,74	2.69	2,82
72	2.27	1,44	2.75	2,51	2,24
96	1.71	1.02	2,22	2,00	1,74
120	1,46	0.73		1,64	1,28
144	1,73	0,50	1,52	1.51	1,31
168	0.92	0,41	1.18	1,14	0,91
192		0,33	0,99	0,93	0,75
216		0.22	0,82	0,79	0,61
240		0.17		0,67	
264			0.46		
288		0,10		0,37	
360		0,06	0,28	0,23	
Dose admin. µg/Kg	483,83	432,09	451,40	406,03	443,34
Weight g.	187	187	179	199	183

Table I : Individual results showing the evolution of ^{14}C radioactivity in the blood of rats as a function of time (see table II) after intramuscular injection of MDI (^{14}C). The results are expressed as a function of the radioactivity injected into the rats, measured per gram of blood (°/$_{oo}$/g). The mean for each period was calculated.

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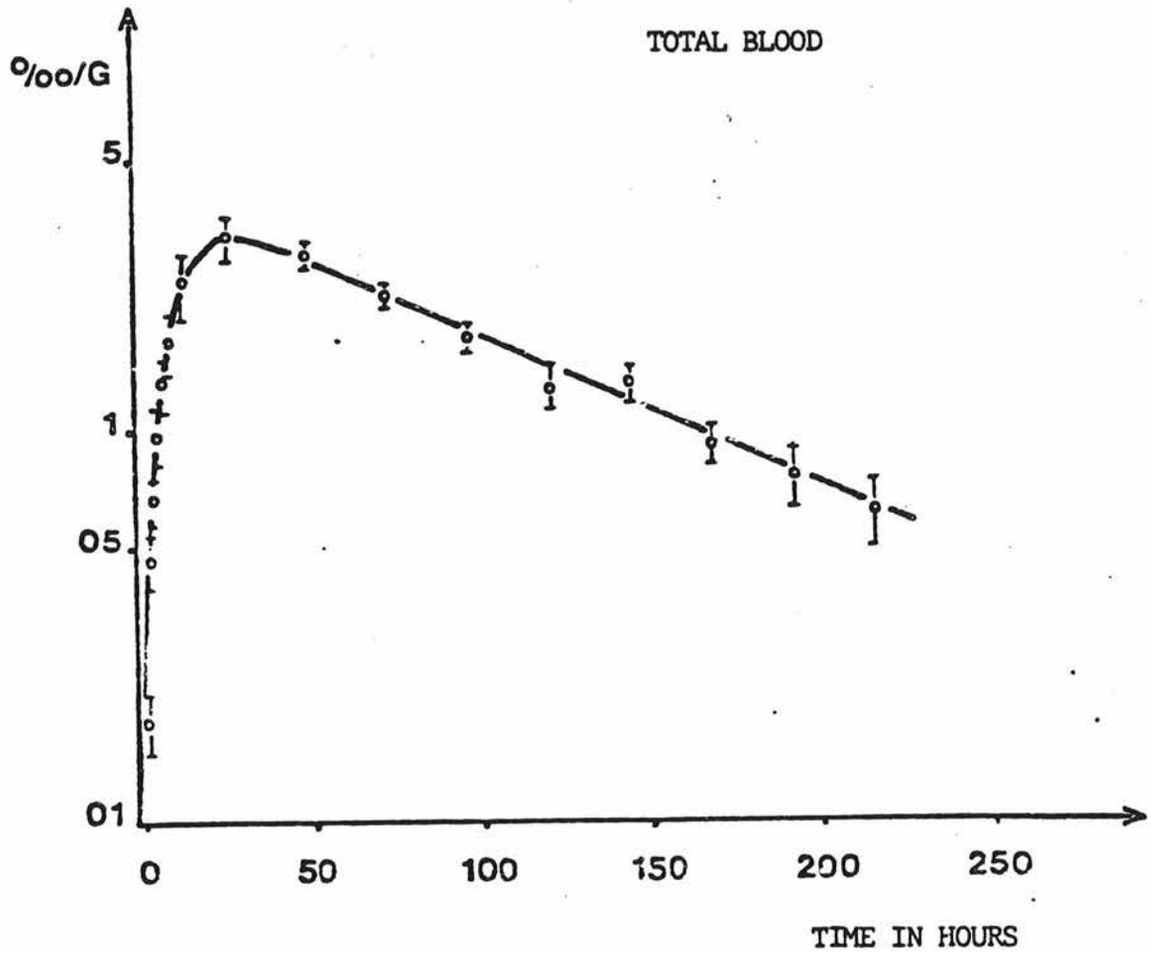

Figure 6 : Variation as a function of time of the logarithm of concentration of radioactivity in the blood of rats having received an intramuscular injection of a single dose of MDI (^{14}C).

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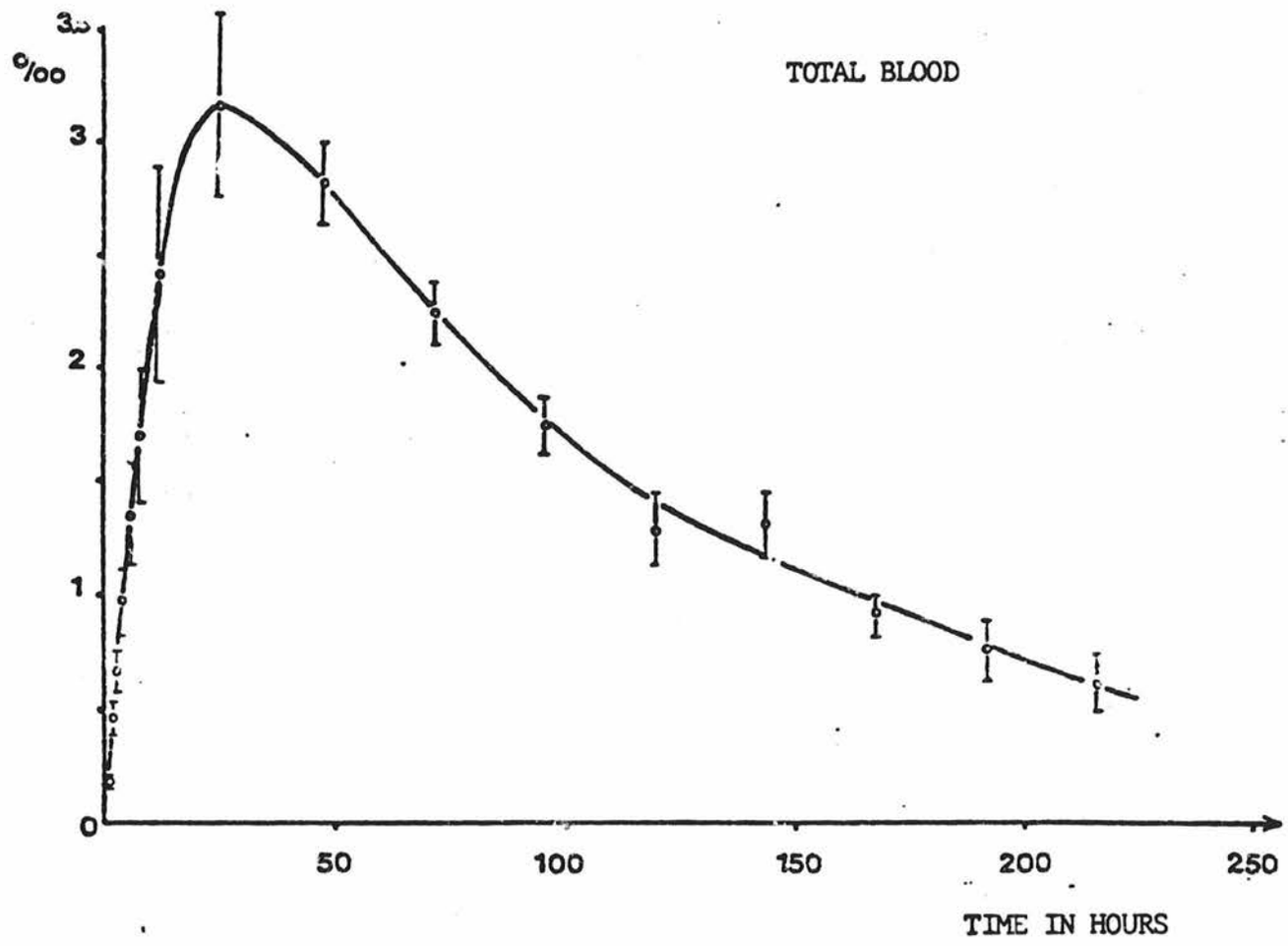

Figure 7 : Variation as a function of time of the concentration of ^{14}C radioactivity in the blood of rats having received an intramuscular injecti of a single dose of MDI (^{14}C).

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The first portion of the curve, which corresponds to increased blood radioactivity, enables evaluation of the diffusion rate of ^{14}C from muscle. After passing through a maximum, the second portion corresponds to the elimination of MDI (^{14}C) and its metabolites from the blood. Although these results are not very accurate, this curve shows that the elimination follows a first order kinetic.

Graphic interpretations and calculations make it possible to estimate the following parameters :

- for elimination : The biological half life for elimination is 78 hou

- for diffusion : The T 1/2 of diffusion from the muscle of injected MDI (^{14}C) is about 12 hours.

b) Urinary and fecal excretion of radioactivity after intramuscular injection of a single dose of MDI (^{14}C).

Table II give the individual results of fractions of radioactivity eliminated during each sampling period. Table III which resume these results make it possible to follow the progress of cumulative quantities of radioactivity deriving from MDI (^{14}C) or its labelled derivatives as a function of time. A check revealed that the elimination of ^{14}C through the respirator tract is not negligible. The ^{14}C compound does not appear as being $^{14}CO_2$. Its fixation by the strong alkaline is bad, in the same experimental conditions $^{14}CO_2$ is bounded with a ratio of 100 %. This observation is improved by the fact that radioactivity in expired air appears after a delay of 2 day after the begining of the experiments.

The curves in figure 8 illustrate the results given in the tables. To summarize, these experiments show the following :

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N° / Period	1 ♂	2 ♂	3 ♀	4 ♀	Mean
URINES					
0 - 6	1.53	0	0	0.22	0.44
6 - 12	3.72	0.46	1.43	2.07	1.92
12 - 24	14.49	7.36	6.89	4.12	8.21
24 - 48	15.75	15.14	9.00	7.90	11.35
48 - 72	11.76	6.79	4.42	4.37	6.83
72 - 96	8.63	3.60	4.35	4.77	5.34
96 - 120	6.03	2.70	2.12	3.00	3.46
FAECES					
0 - 6	0	0	0.06	0	0.01
6 - 12	0.02	0.07	0.22	0	0.08
12 - 24	18.69	13.23	6.62	10.30	12.21
24 - 48	50.45	13.46	40.04	55.51	39.86
48 - 72	40.49	23.14	47.11	44.40	38.78
72 - 96	47.04	12.12	23.84	12.81	23.95
96 - 120	20.67	21.80	22.67	19.28	21.10
WEIGHT g.	188	176	182	193	184.75
Dose admin: µg/Kg	430	459	444	418	437.75

Table II : Individual results showing urinary and fecal elimination of ^{14}C after intramuscular injection of a single dose of MDI (^{14}C) in rats. The results are expressed as a fraction of the dose injected (°/oo). The mean for each period was calculated.

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Period	N°	1 ♂	2 ♂	3 ♀	4 ♀	Mean
URINES						
0 - 6		1.53	0	0	0.22	0.44
6 - 12		5.25	0.46	1.43	2.28	2.35
12 - 24		19.74	7.82	8.32	6.39	10.57
24 - 48		35.49	22.96	17.32	14.29	22.51
48 - 72		47.25	29.75	21.75	18.66	29.35
72 - 96		55.87	33.35	26.09	23.44	34.69
96 - 120		61.91	36.04	28.21	26.43	38.15
FAECES						
0 - 6		0	0	0.06	0	0.01
6 - 12		0.02	0.07	0.29	0	0.09
12 - 24		18.72	13.30	6.91	10.30	10.31
24 - 48		69.16	26.76	46.95	65.81	52.17
48 - 72		109.65	49.90	94.06	110.22	90.96
72 - 96		156.69	62.02	117.90	123.03	114.91
96 - 120		177.37	83.83	140.57	142.31	136.02

Table III : Individual results showing the cumulative urinary and fecal elimination of ^{14}C as a function of time, after administration of a single dose of MDI (^{14}C) by intramuscular injection in rats. The results are expressed in parts per 1000 of the dose injected. The mean for each period as well as the overall balance are calculated.

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Period \ N°	1 ♂	2 ♂	3 ♀	4 ♀
0 - 6	1.91	0.54	0.54	0.44
6 - 12	0.14	0.14	0.15	0.15
12 - 24	0.07	0.22	0.08	0.07
24 - 48	12.70	163.18	13.13	14.73
48 - 72	8.07	12.71	9.13	17.69
72 - 96	0.13	0.10	0.06	0.06
96 - 120	6.10	4.34	2.84	63.89
Total	29.12	181.23	25.93	97.03

Table IV : Individual results showing elimination of ^{14}C versus time through the respiratory path after intramuscular injection of a single dose of MDI (^{14}C) in rats. The results are expressed as a fraction of the dose injected ($^{o}/_{oo}$).

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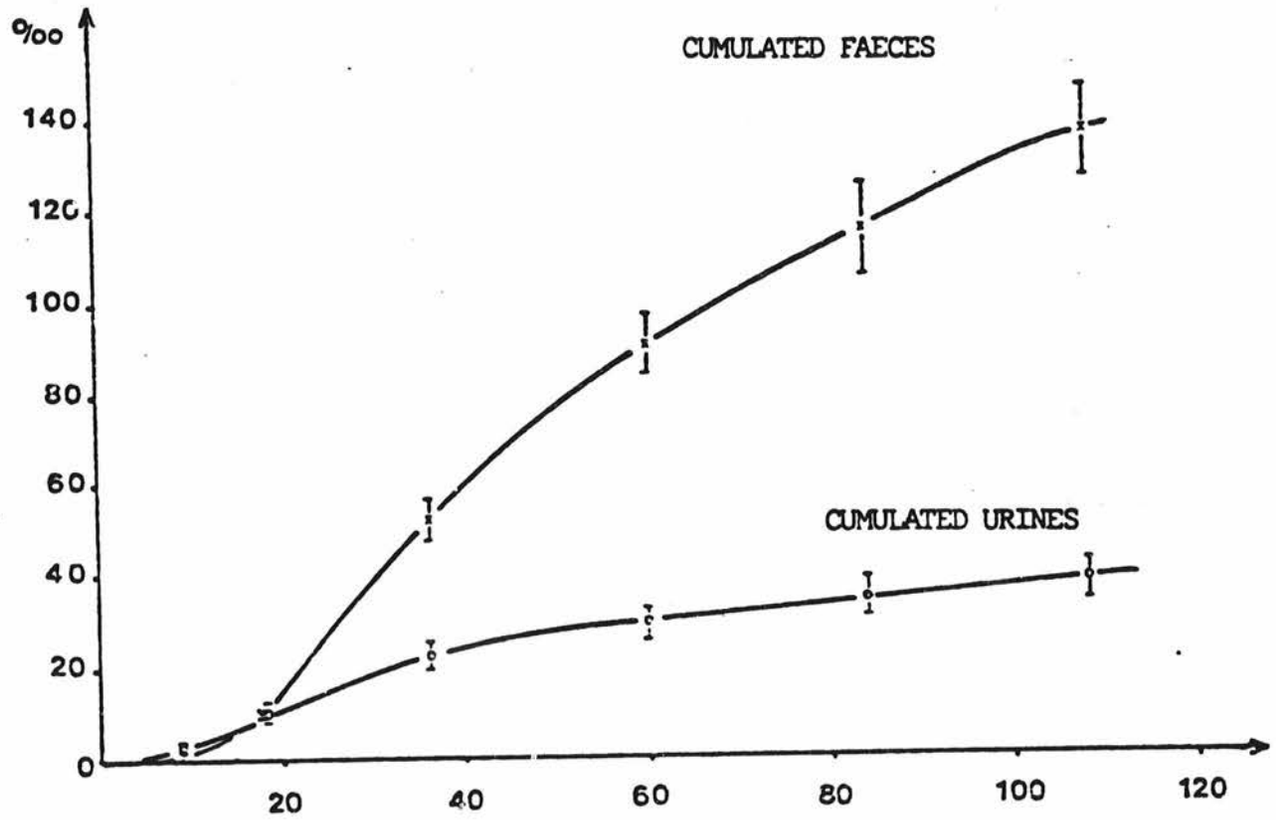

Figure 8 : Curve showing cumulative radioactivities of ^{14}C and its labelled metabolites in faeces and urines as a function of time after intramuscular injection of a single dose of MDI (^{14}C). This curve was plotted from the results given in table III.

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- that the fecal elimination of MDI (^{14}C) and its metabolites labelled with ^{14}C is greater than their urinary elimination (in the ratio of 3.6 to 1)
- that the excreta recovery balance is less than 25 %. The rest is found in the carcases after the animals are killed.

CONCLUSION

The purpose of these experiments was to evaluate the diffusion rate of MDI (^{14}C) from the muscle, before considering contamination of rats via the respiratory tract. This diffusion rate is fairly slow with a T 1/2 of 12 hours. The relatively slow elimination of derivatives of MDI (^{14}C) labelled with ^{14}C should permit observation of blood, urinary and fecal radioactivities with the new experimental procedure.

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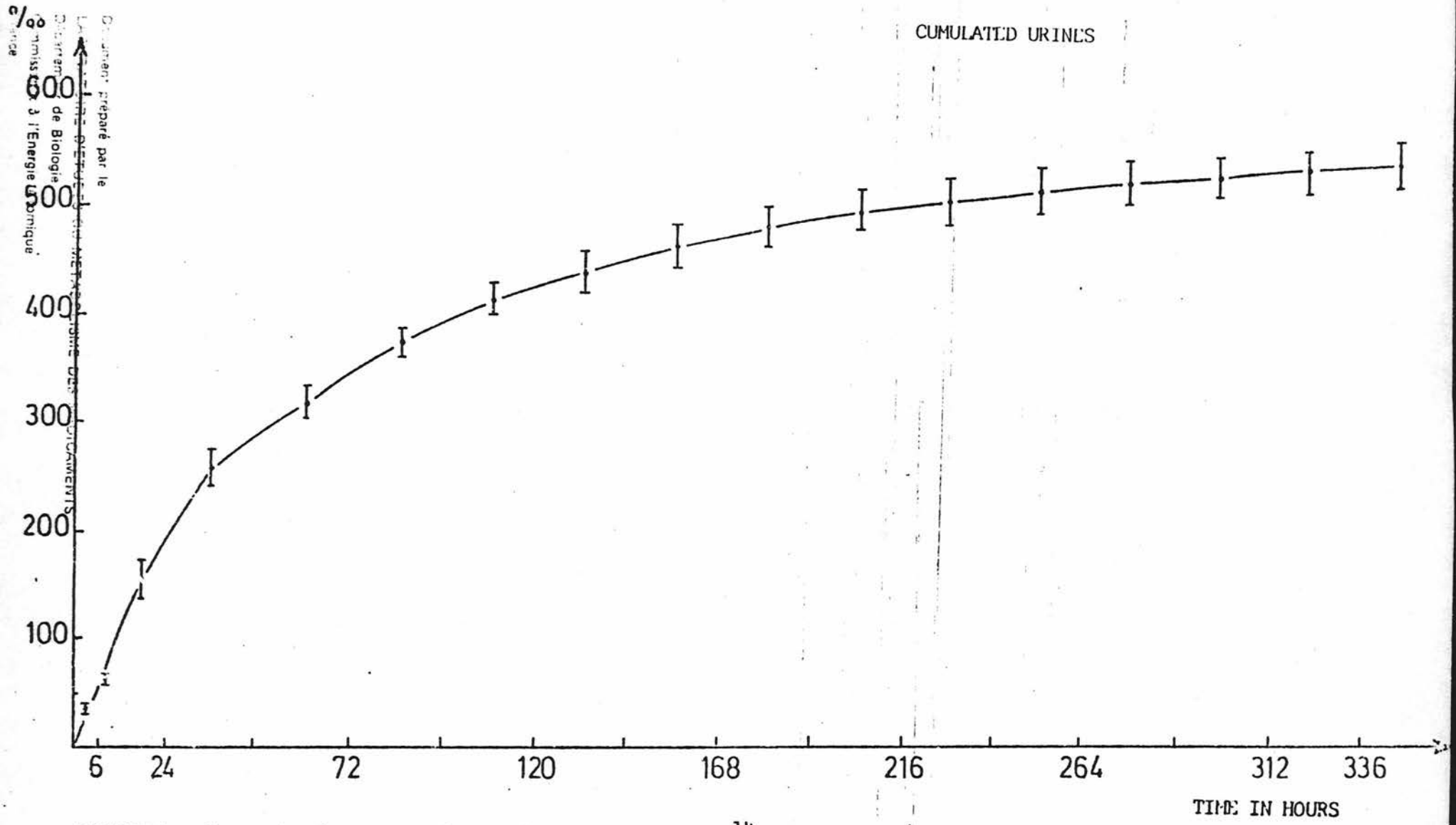

FIGURE 6 : Curve showing cumulative radioactivities of TDI^{14}C and its labelled metabolites in urine as a function of time after intramuscular injection of a single dose of TDI^{14}C. This curve was plotted from the results given in table V.

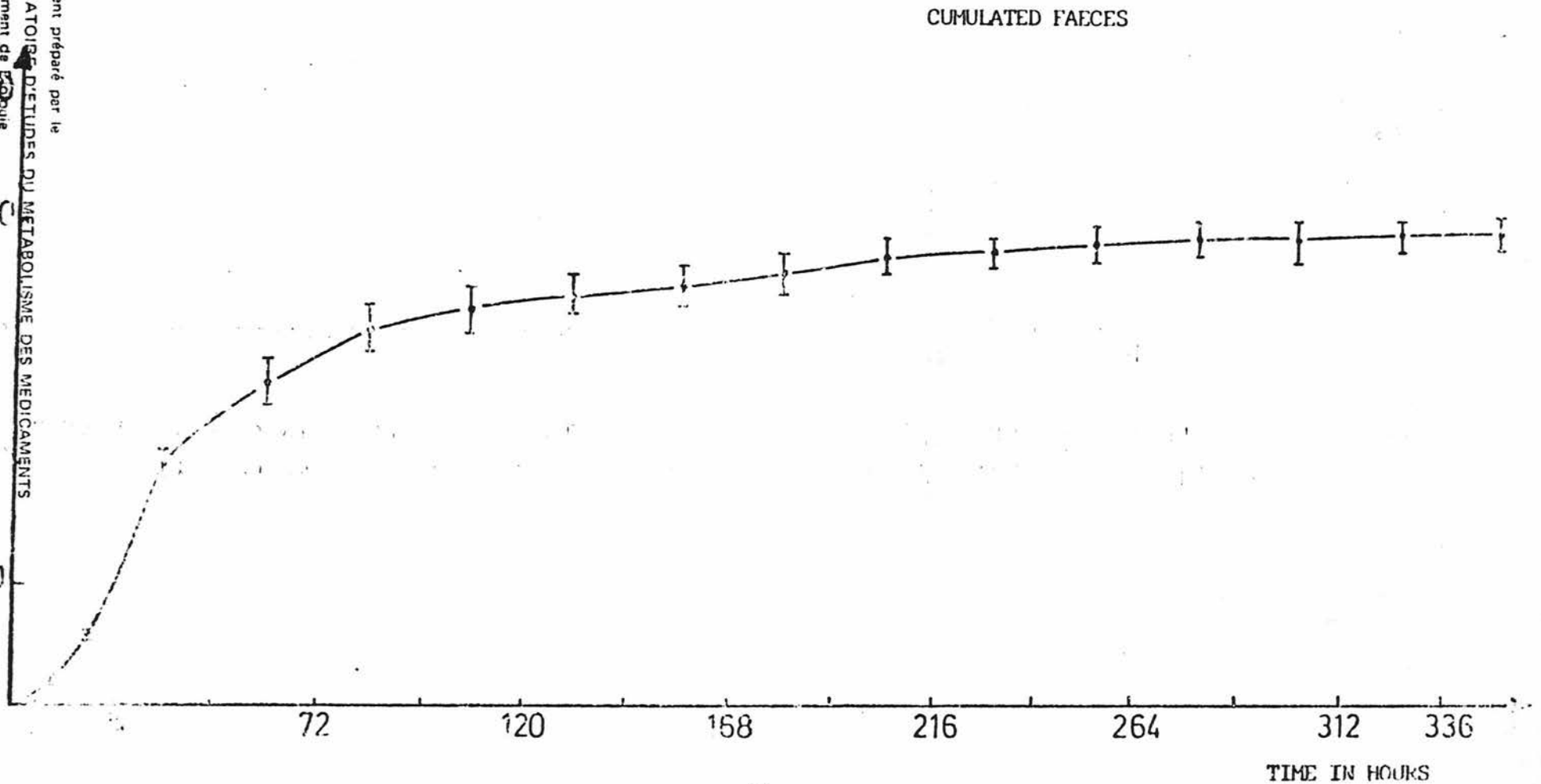

FIGURE 7 : Curve showing cumulative radioactivities of TDI^{14}C and its labelled metabolites in faeces as a function of time after intramuscular injection of a single dose of TDI^{14}C. This curve was plotted from the results given in table VI.

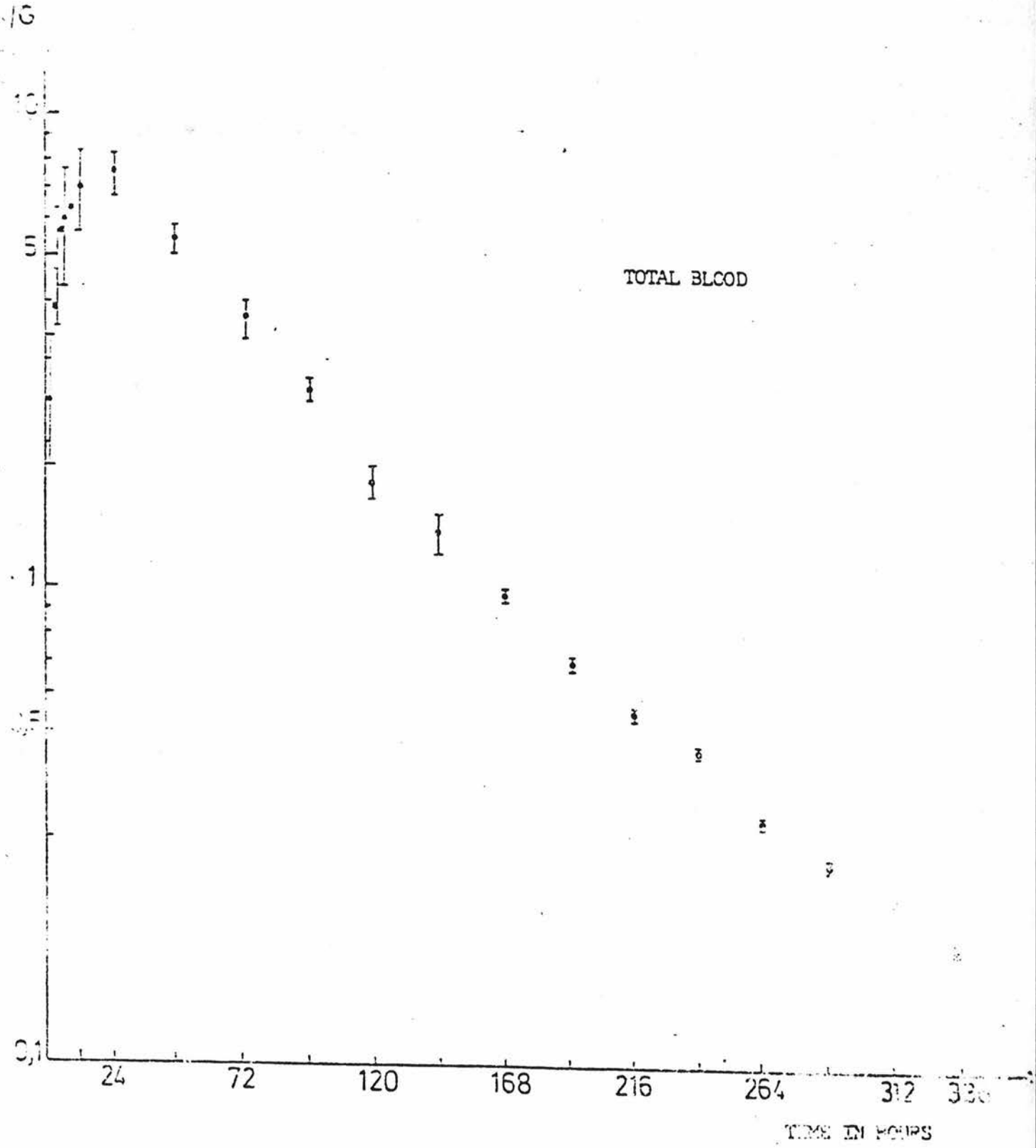

FIGURE 5 : Variation as a function of time of the logarithm of concentration of radioactivity in the blood of rats having received an intravenous injection of a single dose of TDT.(^{14}C).

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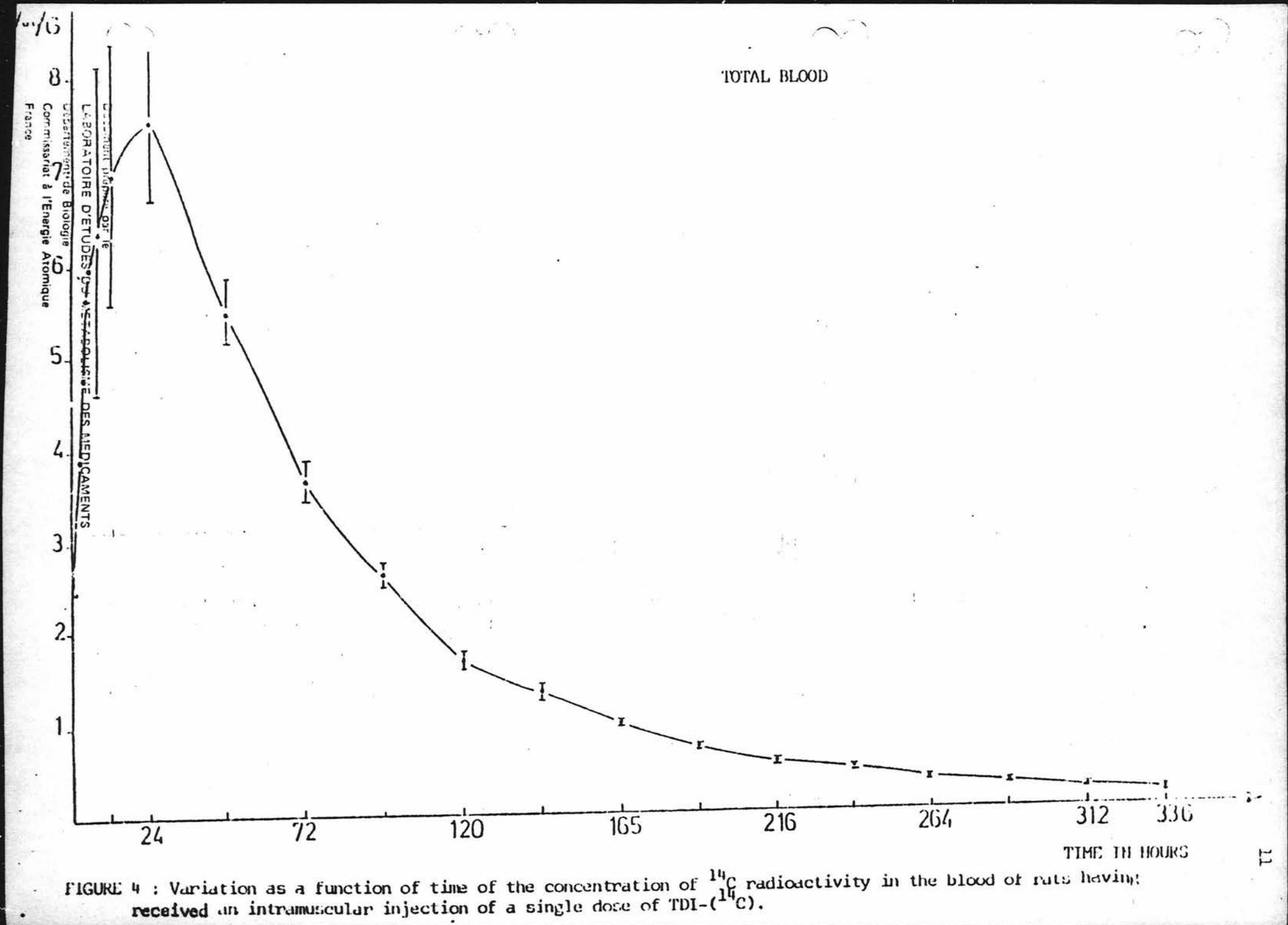

FIGURE 4 : Variation as a function of time of the concentration of ^{14}C radioactivity in the blood of rats having received an intramuscular injection of a single dose of TDI-(^{14}C).

Rat n° / Sex / TIME (h)	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
URINE					
0 - 6	67. 24	4. 59	62. 29	12. 12	38. 06 ± 7. 85
6 - 12	31. 96	18. 69	28. 17	22. 49	25. 33 ± 1. 47
12 - 24	158. 20	104. 08	59. 34	48. 11	92. 43 ± 12. 53
24 - 48	91. 16	96. 23	76. 35	144. 81	102. 15 ± 7. 42
48 - 72	45. 13	76. 07	46. 11	75. 03	60. 58 ± 4. 32
72 - 96	27. 03	59. 72	45. 76	79. 79	53. 07 ± 5. 57
96 - 120	38. 70	62. 19	32. 66	24. 86	39. 60 ± 4. 02
120 - 144	29. 78	51. 32	28. 82	35. 61	36. 38 ± 2. 60
144 - 168	15. 78	33. 95	11. 22	30. 35	22. 82 ± 2. 76
168 - 192	12. 22	26. 55	16. 80	17. 66	18. 31 ± 1. 50
192 - 216	11. 66	16. 58	12. 51	12. 25	13. 26 ± 0. 56
216 - 240	10. 56	9. 30	11. 02	9. 35	10. 06 ± 0. 22
240 - 264	6. 95	9. 03	10. 15	6. 10	8. 81 ± 0. 33
264 - 288	6. 56	8. 14	6. 75	10. 57	8. 00 ± 0. 46
288 - 312	5. 40	4. 96	3. 47	4. 92	4. 69 ± 0. 21
312 - 336	4. 14	4. 76	3. 56	3. 38	3. 96 ± 0. 13
336 - 360	2. 68	3. 36	4. 46	1. 38	2. 97 ± 0. 32

TABLE III : Individual results showing urinary elimination of ^{14}C after intramuscular injection of a single dose of TDI ^{14}C in rats (see table VII). The results are expressed as a fraction of the dose injected ($^{o}/_{oo}$). The mean for each period was calculated.

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- a ^{14}C compound (TDI^{14}C or one metabolite) is distributed in two compartments in the organism,
- we are in the presence of two labelled derivatives, metabolites with different elimination kinetic parameters.

Graphic interpretations and calculations make it possible to estimate the following parameters :

- for elimination : the relative magnitudes of the two components of the elimination curve are in a ratio of about 3.8.

The half-lives of the two components are 32 hours for the larger compartment and 73 hours for the smaller.

- for diffusion : the T 1/2 of diffusion from the muscle of injected TDI^{14}C is about 30 minutes.

b) Urinary and fecal excretion of radioactivity after intramuscular injection of a single dose of TDI^{14}C.

Tables III and IV give the individual results of fractions of radioactivity eliminated during each sampling period. Tables V and VI, which resume these results, make it possible to follow the progress of cumulative quantities of radioactivity deriving from TDI^{14}C or its labelled derivatives as a function of time. A check revealed that the elimination of ^{14}C in the form of $^{14}CO_2$ through the respiratory tract is negligible, showing that, in rats, there is no transformation of the molecule by demethylation of the radical $-^{14}CH_3$.

The curves in figures 6 and 7 illustrate the results given in the tables. To summarize, these experiments show the following :

- that the urinary elimination of TDI^{14}C and its metabolites labelled with ^{14}C is greater than their fecal elimination (53 % as compared with 39 %),
- that the excreta recovery balance is better than 92 %. Four per cent is found in the carcases after the animals are killed.

CONCLUSION

The purpose of these experiments was to evaluate the diffusion rate

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Rat n° / Sex / TIME (h)	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
URINE					
0 - 6	67. 24	4. 59	62. 29	18. 12	38. 06 ± 7. 84
6 - 12	99. 20	23. 28	90. 46	40. 62	63. 39 ± 9. 29
12 - 24	257. 40	127. 36	149. 79	88. 73	155. 82 ± 18. 06
24 - 48	348. 56	223. 63	226. 15	233. 55	257. 97 ± 15. 13
48 - 72	393. 69	299. 71	272. 26	308. 58	318. 56 ± 13. 10
72 - 96	420. 72	359. 43	318. 02	388. 36	371. 63 ± 10. 91
96 - 120	459. 42	421. 62	350. 68	413. 22	411. 23 ± 11. 27
120 - 144	489. 20	472. 94	346. 83	448. 83	439. 45 ± 15. 98
144 - 168	504. 98	506. 89	358. 05	479. 18	462. 27 ± 17. 65
168 - 192	517. 19	533. 43	374. 85	496. 84	480. 57 ± 18. 01
192 - 216	528. 85	550. 01	387. 37	509. 10	493. 83 ± 18. 23
216 - 240	539. 41	559. 32	398. 38	518. 45	503. 89 ± 18. 07
240 - 264	546. 36	568. 35	408. 53	524. 55	511. 95 ± 17. 81
264 - 288	552. 92	576. 49	415. 28	535. 12	519. 95 ± 17. 95
288 - 312	558. 31	581. 45	418. 75	540. 04	524. 64 ± 18. 15
312 - 336	562. 45	586. 21	422. 31	543. 42	528. 59 ± 18. 25
336 - 360	565. 13	589. 57	426. 77	544. 80	531. 57 ± 18. 05

TABLE V : Individual results showing the cumulative urinary elimination of ^{14}C as a function of time, after administration of a single dose of TDI ^{14}C by intramuscular injection in rats (see table VII). The results are expressed in parts per 1000 of the dose injected. The mean for each period as well as the overall balance are calculated.

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Rat n° / Sex / TIME (h)	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
FAECES					
0 - 6	0	3. 64	0	0	0. 91 ± 0. 45
6 - 12	26. 56	18. 45	9. 23	23. 93	19. 54 ± 1. 92
12 - 24	54. 09	33. 09	18. 25	45. 99	37. 85 ± 3. 92
24 - 48	150. 19	94. 26	215. 08	109. 15	142. 17 ± 13. 51
48 - 72	58. 49	66. 75	117. 59	59. 99	75. 70 ± 7. 04
72 - 96	34. 07	38. 38	43. 54	23. 32	34. 83 ± 2. 15
96 - 120	17. 41	20. 75	14. 21	15. 79	17. 04 ± 0. 70
120 - 144	11. 35	15. 57	7. 99	12. 61	11. 88 ± 0. 78
144 - 168	6. 58	11. 99	7. 02	11. 41	9. 25 ± 0. 71
168 - 192	6. 65	11. 32	8. 71	11. 71	9. 60 ± 0. 59
192 - 216	6. 26	7. 74	4. 12	13. 04	7. 79 ± 0. 95
216 - 240	4. 76	10. 67	3. 40	12. 90	7. 93 ± 1. 14
240 - 264	3. 39	8. 48	3. 58	11. 07	6. 63 ± 0. 95
264 - 288	3. 69	4. 89	2. 84	6. 07	4. 37 ± 0. 35
288 - 312	1. 83	4. 21	0. 58	5. 46	3. 02 ± 0. 55
312 - 336	1. 77	2. 44	1. 49	3. 19	2. 22 ± 0. 19
336 - 360	1. 99	1. 65	1. 56	1. 36	1. 64 ± 0. 07

TABLE IV : Individual results showing fecal elimination of ^{14}C after intramuscular injection of a single dose of TDI ^{14}C in rats (see table VII). The results are expressed as a fraction of the dose injected (‰). The mean for each period was calculated.

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N°	1	2	5	6
SEX	♂	♂	♀	♀
Weight in g:	157	150	139	134
Dose TDI Admistered µg/animal	17.57	17.57	32.54	23.11

TABLE VII : Individual doses of TDI administered to animals used for excretion balances.

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TIME (h) \ Rat n° / Sex	1 ♂	2 ♂	5 ♀	6 ♀	MEAN ± es
FAECES					
0 - 6	0	3.64	0	0	0.91 ± 0.45
6 - 12	26.56	22.09	9.23	23.93	20.45 ± 1.93
12 - 24	80.66	55.18	27.48	69.92	58.31 ± 5.76
24 - 48	230.84	149.43	242.56	179.07	200.47 ± 10.95
48 - 72	289.33	216.18	360.15	239.05	276.17 ± 15.94
72 - 96	323.41	254.56	403.69	262.38	311.01 ± 17.26
96 - 120	340.82	275.30	417.90	278.17	328.05 ± 16.77
120 - 144	352.17	290.88	425.90	290.78	339.93 ± 16.04
144 - 168	358.75	302.87	432.92	302.19	349.18 ± 15.45
168 - 192	365.40	314.18	441.63	313.90	358.77 ± 15.08
192 - 216	371.66	321.92	445.76	326.95	366.57 ± 14.33
216 - 240	376.42	332.59	449.16	339.85	374.51 ± 13.33
240 - 264	379.80	341.07	452.74	350.92	381.13 ± 12.62
264 - 288	383.49	345.96	455.57	356.99	385.50 ± 12.32
288 - 312	381.64	350.17	456.15	362.45	387.60 ± 11.87
312 - 336	383.40	352.61	457.64	365.64	389.82 ± 11.73
336 - 360	385.39	354.26	459.20	367.00	391.46 ± 11.73
TOTAL URINE + FAECES	950.52	943.83	885.97	911.80	923.03 ± 7.48

TABLE VI : Individual results showing the cumulative fecal elimination of ^{14}C as a function of time, after administration of a single dose of TDI ^{14}C by intramuscular injection in rats (see table VII). The results are expressed in parts per 1000 of the dose injected. The mean for each period as well as the overall balance are calculated.

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APPENDIX I

AUTORADIOGRAPHY OF THE ENTIRE ANIMAL

PROCEDURE : A rat weighing 100 g receives an intramuscular injection of TDI^{14}C (18 µCi). Twenty four hours after administration of the chemical, the animal is killed and rapidly frozen by immersion in liquid nitrogen. The frozen rat is stored for 24 hours in a freezer at -25°C before the preparation of sections. Ullberg's technique (1954) was employed for preparing the sections. A leitz microtome was used with a plate cooled to -30°C.

50 µ sections parallel to the sagittal axis of the animal were prepared. Collected on adhesive tape, they were dehydrated and then placed against a "Kodirex" monolayer (Kodak) radiological film. The films were developed after five days of exposure. The black areas enable localization of the radioactivity due to ^{14}C introduced into the body of the animal in the form of TDI^{14}C.

RESULTS

Plate 1 shows the autoradiographs of a rat killed 24 hours after administration of the labelled medicament. This period corresponds approximately to the activity peak in the plasma of the animal.

The relative importance of tissue fixation of the chemical is shown in table I. These patterns were obtained from preparations of the same thickness (50 µ). The exposure period was seven days in all cases.

REFERENCE

Ullberg S., 1954, Acta Radiol. Supp. 118

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of TDI^{14}C from the muscle, before considering contamination of rats via the respiratory tract. This diffusion rate is fairly rapid with a T 1/2 of 30 minutes. Our experience suggests that diffusion from the respiratory tract should be even more rapid. Moreover, the relatively slow elimination of derivatives of TDI^{14}C labelled with ^{14}C should permit observation of blood, urinary and fecal radioactivities with the new experimental procedure.

The autoradiograph of the entire animal (see Appendix) shows that TDI^{14}C and its labelled metabolites are practically diffused throughout the organs, with a high concentration in the excretion organs.

From the purely kinetic standpoint, the distribution curves based on distribution in blood and on the excretion balance suggest that in addition to TDI, metabolites are formed :

- one or two important metabolites diffusible through the majority of organs :
  - with relatively slow elimination rates,
  - with an elimination balance approaching 100 % in 15 days.

These hypotheses will only be corroborated by additional investigations

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